

Third Revised Edition

An Introduction to
Viruses

S B Biswas • Amita Biswas

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AN INTRODUCTION TO VIRUSES

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An Introduction to **VIRUSES**

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PREFACE TO THE THIRD REVISED EDITION

A revision of the first edition was overdue. For one thing the original text needed some elaboration, re-organization and updating. For another, some acts of commission and omission which crept in inadvertently in the earlier text, needed to be remedied.

Briefly, the existing content has been re-arranged with a view to accommodating additional information and recent concepts. The basic format has not been altered, however. Emphasis has been laid on general techniques related to physical, chemical and biological characteristics of viruses; genetics of viruses have been discussed separately as has been their physiology. In general, the text has been kept at an introduction level of presentation. Details have been avoided unless specifically required pertaining to a major development or to a particular phenomenon. Applied aspects of virology have also been covered. An appendix has been added highlighting some significant recent developments in virology. Glossary, bibliography and indices complete the text.

It is indeed a pleasure to record our appreciation of the heartening response received by the first edition. Coming from students and teachers alike, these have acted at once as a reward and as a source of encouragement. Improvements suggested and mistakes pointed out by readers have helped us greatly in compiling the revised text.

One discordant note which we are constrained to strike relates to the status of general virology as a discipline in the curricula of the Indian universities. It is indeed sad to reflect that virology is yet to receive its due; our syllabuses in the biological sciences remain heavily loaded in favour of classical botany and zoology, microbiology taking a distant back seat. And all this despite the recent emphasis being paid by the government towards the development of biotechnology and despite the fact that we are fast approaching the 21st century which is likely to usher in a biological revolution vis-a-vis the human civilization. One can only hope that attitudes will change for the better.

The revised edition is meant to be an improvement over the first. However, as we had occasion to mention earlier also, there is always a scope for further improvement. Suggestions in this respect are warmly welcome.

AUTHORS

PREFACE TO THE FIRST EDITION

A book of general virology, meant especially for degree students, perhaps needs a few words by way of explanation. To be very precise, we felt that such a text was long overdue and, if carefully brought out, should go a long way in meeting a long-felt need.

It need not be mentioned that this branch of knowledge has expanded at a fantastic rate during the last couple of decades; and that it is continuing to do so at an increasing pace. New data have been unearthed, new concepts have emerged. The sheer volume of information and ideas, when put in print, is staggering.

It should be reasonable to expect, therefore, that our students are well acquainted with the basic features of the expanding horizons of virology. Unfortunately, this had not been the case, particularly at the degree level. The subject has been tended to be taught piecemeal and ideas about viruses have remained vague and incomplete.

We feel that there are two main reasons responsible for the prevailing situation. For one thing, university syllabuses have remained unimaginative and stagnant. Curiously enough, this branch of life science has remained outside the curricula prescribed for Zoology courses of our universities. Even in Botany, where it is taught, the scope and purview have remained rather limited. Lack of reasonably up to date texts, cheap enough to be within the reach of our students, has been the other responsible factor. The experience of one of us in teaching the subject to undergraduate students for the last many years, bears testimony to all this. The situation obtaining prompted us to give shape to this project which has been there on our minds for quite some time.

A few words about the book, we feel, will not be out of place. It was designed with a view to providing a comprehensive but generalised account of viruses. Necessarily, therefore, the formation of chapters and the treatment of the material has been subjective to some extent. The book begins with an introductory chapter followed by chapters on the physico-chemical nature, taxonomy and phylogeny, process of replication and genetics of viruses. These are followed by chapters on host-virion interrelationships, viral diseases, their transmission and control, and the importance of viruses to human civilization. A glossary of relevant terms used in the text has been provided.

with to assist the reader in pursuing the text. A list of suggested readings has also been appended.

The book does not purport to be a reference text. It is possible, therefore, that certain important but detailed informations have been left out. There may also have been a few inadvertent acts of omission and commission. Despite all these, we hope that the book will elicit proper response from the students and the teachers reading the book.

We believe in the dictum that there is always a scope for improvement. We, therefore, earnestly solicit all suggestions sent to us with this objective. After all is said and done, we hope this book will serve some fruitful purpose to our students for whom it is ultimately meant.

AUTHORS

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HISTORICAL INTRODUCTION

The term, *Virus* has originated from a Latin word which means poison. To a layman it conveys a similar impression. This word is always associated with some disease or the other. The word has been and is still being used often to describe unpleasant and harmful influences; we also talk about virus in one's mind.

In a biological sense, the word has been in use for about a hundred years. Scientists first applied the word to describe such agents which they thought to be responsible for causing certain diseases; agents which they found mysterious and dangerous but which could not be placed within the framework of existing knowledge about the living world. The term gradually came to signify a special type of entity whose properties were found in many respects to be completely different from those of living organisms. Information about these substances were gradually gathered and a whole branch of study was developed. This branch of study is referred to as *Virology* and is today one of the most important of the sciences. People dealing with the subject are called *Virologists*. They are engaged in numerous laboratories all over the world, busy in gathering newer and newer information about viruses and in developing fresh ideas regarding them.

The viruses had been with us and are with us all the time. Sometimes they are harmful while sometimes they are not. But they are no more the mysterious and dreadful agents which they were once considered to be. Continuous efforts by virologists have changed our opinion about them. Emergence of certain concepts has helped us in understanding them in a better manner than we could do previously. And also, perhaps more important than that, now we are more knowledgeable about and more confident in facing problems concerning or arising out of viruses.

EARLY RECORDS

History records several incidences involving, what we now know to be, viruses. Diseases caused by viruses have been recorded by ancient civilizations like the Indian or the Chinese. Small-pox, influenza and

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common cold are particularly mentioned in ancient literature. In fact, some diseases, like the common cold, were so common that they were not seriously considered as diseases. However, people were very much afraid of the more harmful of these diseases, namely, small-pox, etc. They linked those diseases to unnatural and supernatural causes. *Pujas* and offerings were performed to please the gods and goddesses, thought to be responsible for bringing about the diseases. Goddess *Sitala* was considered to be responsible for small-pox and worshipped as such by millions of our countrymen. Though this practice of worshipping is quite old but its origin is not yet clear. The idea, of course, is to propitiate the goddess so that she does not harm her worshippers. In the remote villages of our land the practice still continues; and the people there are, in fact, more faithful towards the goddess than they are to modern science. There was a time when people were afraid even to get vaccinated against small-pox. They thought it might annoy goddess *Sitala* and harm them even more. Such superstition was common to people in almost every part of the world. Ignorance and fear ruled the minds of the people. Knowledge about viruses or, for that matter, about any other disease causing organism was either very little or nonexistent. Organised science was still to gain an upper hand.

People used to die in large number due to these diseases. According to one estimate, sixty million people died of small-pox alone during the eighteenth century. In India, this disease used to occur with a definite regularity. Earlier, every time it occurred it resulted in a substantial number of deaths. With the adoption of stringent precautionary measures in recent years, like mass vaccination, the casualty rates have been lowered to a considerable extent. However, in spite of this, epidemics sometimes occur. The epidemic which occurred in Bihar in 1973-74 is still fresh in our minds.

Of the other viral diseases, influenza used to claim lives in millions. It still occurs in epidemic form all over the world. Yellow-fever, which is primarily a tropical disease, used to cause death in thousands in tropical countries of Central America and Africa. Sailors from temperate European countries used to fear this disease. This fear was quite natural in view of the sudden appearance of the disease often resulting into the death of shiploads of sailors. Mysterious disappearance of ships such as the Flying Dutchman is thought to be due to a disaster caused by this disease.

One interesting historical phenomenon involving viruses and plants deserves to be mentioned. This is not so much because of the disease that is caused to the plant but because of its sociological significance. The plant concerned is the tulip which is an ornamental plant cultiva-

ted for its beautiful flowers. In the sixteenth and the seventeenth centuries a variety of tulip was considered very attractive and, therefore, was very popular. This was because of the presence of different shades or striations on the petals of these flowers. Such shaded tulips were called 'broken' tulips meaning that these had 'breaks' or gaps in their normal colourations. In those days the reasons for such 'broken' appearance were not known. Much later it came to be known that such 'breaks' developed as a result of infection by a virus.

People in France and Holland were particularly crazy about 'broken' tulips. Cultivation and possession of these were immensely prized and regarded as status symbols. There are stories relating as to how brides possessing bulbs of such tulips were considered very much desirable by prospective bridegrooms. Apparently these ladies were earnestly sought after by the gentlemen concerned and had all the difficulty in choosing their husbands. The craze for tulips or 'tulipomania' is, also, a thing of past.

Although some information regarding the aforementioned viral diseases had been obtained in course of time, nothing was known about the reasons that caused such diseases. It was of course known that these diseases were infectious and were transferred from the diseased person to the healthy ones. It was known, for instance, that dried scales of small-pox rashes if rubbed onto the limbs of healthy persons saved them from getting infected subsequently. In fact it is reported to have been practised by *vaid*s in ancient and mediaeval India. Similarly, remedy against common cold was prescribed often successfully. However, these practices could at best be regarded as precursor to the later developments. A few preventive measures and remedies were probably discovered and used. This was the situation almost upto the end of the eighteenth century. In 1796 Dr Edward Jenner discovered vaccination against small-pox. At that time this discovery could not be linked to viruses or to any other living organism. Nevertheless, this event should be regarded as an important milestone in the development of the science of virology. In any case lives were saved and that was important.

At about the same time it was also known that the phenomenon of 'tulip break' could be transferred from individuals of one variety to those of another. It was observed that such transfer was not possible with most other types of plants showing similar shades or striations on their flower petals. Evidently, tulip 'break' was considered something unique. One Mr Henry Cane, a cultivator of tulips and other flowers noted, with reference to tulip 'break' and Jessmine 'mottle' (a related phenomenon), "I have tried other sorts of variegated plants

but do not find any of them transmute as tulip or jassamine will do." Obviously, this transfer was similar to the transfer of infectious diseases. No idea was there as to how such transfer was possible.

SCIENTIFIC INVESTIGATIONS

By the middle of the nineteenth century investigation into the causes of infectious diseases was being carried with more seriousness than ever before. Infectious nature of many diseases were established. The real breakthrough came in the sixties of that century. In 1865, Louis Pasteur was able to demonstrate that it was a protozoa that caused a serious disease to silkworms thus harming the silk industry in France. Anthrax of sheep was another serious disease that was harming the economy of several European nations at that time. In 1876 Dr Robert Koch, a German Bacteriologist, demonstrated that a bacterium was invariably present in the blood of the infected sheep. He also showed that these bacteria could be isolated and cultured under laboratory conditions and that these were able to cause disease in healthy individuals even after that. Louis Pasteur was working in France on the same problem at that time. His conclusions were similar to those of Koch. These two scientists firmly established the idea that infectious diseases were caused by some micro-organism or the other. They were thus instrumental in developing what has come to be known as the 'germ theory' of disease.

Although 'germ theory' was able to account for a large number of diseases, particularly those caused by the bacteria, the occurrence of a few important ones could not be satisfactorily explained. For example, the problems of small-pox and influenza remained unsolved. Scientists of the day investigating these diseases were quite puzzled. Could it be that these were not caused by bacteria or by any other micro-organism? If that were so, what could possibly cause them? These and other similar questions were uppermost in their minds.

At about the same time Pasteur was investigating the disease *rabies* in all its aspects. He was most bothered about the problem concerning its casual agent. He was able to demonstrate that the disease was infectious. But despite repeated attempts he was not successful in establishing the nature, the agent responsible for causing and spreading it. He was almost certain that bacteria were not responsible for causing the disease. However, he was not so sure about the presence of any other types of organism that could cause it. He, therefore, had to remain satisfied by suggesting that some undefinable 'sub-microscopic' (that which cannot be seen microscopically) organism could be respon-

sible for causing rabies.

During those days one important infectious disease of plants, known as the *mosaic disease of tobacco*, was causing serious damage to the tobacco crop in Holland and Germany. It was being investigated from different angles in these two countries. Adolf Mayer, a German Botanist, reported in 1886 that this disease could be transmitted by just applying the sap or juice of infected leaves onto the leaves of healthy individuals. However, he could not see any bacteria in the clarified saps. Nor could he isolate any bacterium from them. Nevertheless, he recorded two important findings. In the first place, he noted that the intensity of infection remained unchanged even after partial purification of the sap. Secondly, he found that the capacity to infect was considerably destroyed when the clarified sap was heated and that above 55°C the loss was almost total.

Significance of these observations was not properly understood by Mayer. He continued to search for some bacteria or the other in the sap as he was convinced about their presence. One reason for his belief was, quite wrongly of course, his observation that infectivity was lost when the sap was passed through a pad of filter papers having very small pore size. It was known that bacteria because of their larger size could not pass through such a pad. Mayer therefore concluded that the sap passed through this type of filter could not contain any bacteria. He was right in making this conclusion. However, his observations that the capacity to infect was lost, was incorrect. Therefore, his final inference that the supposed loss in infectivity could be linked to the absence of bacteria in the filtered sap was wrong.

DISCOVERY

A few years after a most remarkable discovery was made by a Russian botanist named Dimitry Iwanowski. He was also engaged in the investigation of mosaic disease of tobacco. Particularly he was trying to find out the nature of the agent causing the disease. He repeated most of Mayer's experiments and confirmed some of his (Mayer's) results. For example, he also found that the sap obtained from the leaves of infected plants was capable of causing fresh infection in healthy plants. He also demonstrated that the power to infect was lost if the sap was previously heated. He, however, strongly rejected Mayer's observation that the capacity to infect was lost when the sap was passed through a pad of filter papers. He reported that infectivity was retained not only on filtration of the sap through a pad of filter papers but even when the sap was passed through more sophisticated filters.

One of such filters commonly used in those days was the Chamberland-Roux filter. It was named after its main discoverer Dr Chamberland who was a well-known bacteriologist of his time. These filters have extremely fine passages which allow not even the smallest of bacteria to pass through. Iwanowski repeatedly found the same results and forcefully claimed that the agents responsible for causing infection could pass through filters that would not allow bacteria to pass through. He called these agents *Filtrable* which indirectly meant that these were of a nature different from bacteria. However, Iwanowski could not think about a new type of organism altogether. He simply hinted at the possible existence of bacteria smaller than they were known to at that time. He even considered them submicroscopic (beyond the vision of microscopes). This was in 1892.

CONTAGIUM VIVUM FLUIDUM

At that time M.W. Beijerinck, a Dutch microbiologist got interested in these filtrable agents causing the mosaic disease of tobacco. He confirmed Iwanowski's findings and noted the filtrable nature of the agent responsible for the disease. However, he was not at all convinced by Iwanowski's suggestion that these agents were submicroscopic particles. His idea was that these were not particulate at all. Rather he considered them to be fluid in nature. He performed a very simple but ingenious experiment which convinced him that these were indeed so (Fig.1.1).

He prepared blocks of solidified agar (gelly like substance of polysaccharide nature used as base for cultivating micro-organisms in the laboratory; obtained from some red alga) of suitable sizes. He then spread the sap of infected tobacco leaves on these blocks and left them as such for a time. His idea was that the liquid portion of the sap would diffuse through the agar and would ultimately be present in the lower regions as also in the upper regions of the agar block. However, if bacteria were present in the sap they would not be able to move through the agar rather because of their particulate nature, these would remain aggregated as a separated layer on top of the block.

After the required duration of time, he took the sap saturated agar blocks and removed a thick upper layer. He then injected a suspension of the remaining portion of the agar block into healthy tobacco plants. The disease duly appeared. This showed that the filtrable agent was diffusible through the agar medium and, therefore, was probably liquid in nature. Beijerinck had repeated this experiment many times and was very sure that the filtrable agents causing mosaic of tobacco were liquid indeed. In 1898 he put forward a new concept

regarding these agents. He called them *Contagium Vivum Fluidum* or 'living infection fluid.' This idea was considered to be revolutionary because it for the first time acknowledged the possibility of a new type of disease causing agent which was so very different from those that were known at that time.

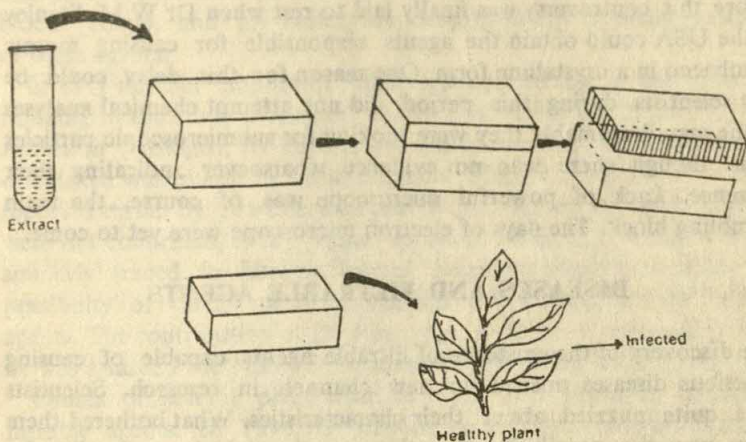


FIG. 1.1. A diagrammatic representation of Beijerinck's experiment designed to show that the 'filtrable agents' causing the mosaic disease of tobacco could diffuse through agar medium. The agents were taken in a suspension and poured onto an agar block. After some time the upper portion of the block was removed. The lower half was made into a suspension and inoculated into a healthy tobacco plant. Disease duly developed proving that the agents could diffuse through agar block. It also showed that the disease was not caused by bacteria. Because bacteria would have been retained at the top of the block and would not have diffused through. The filtrable agents were not, therefore, bacteria.

Despite all these developments, however, the controversy regarding these agents was not over. Rather, it started in a new direction. In the year Beijerinck proposed this new concept, two German scientists, Drs Loeffler and Frosch had suggested that these filtrable agents were particles of a new type. They were searching for the casual agent of foot-and-mouth disease of cattle during those years. This was a serious disease affecting cattle and was causing considerable financial loss to the farmers. They found that the casual agents of this disease also were filtrable in nature. However, they regarded these to be particle like or corpuscular. Beijerinck was opposed to their ideas. He "cannot agree with Dr Loeffler as regards the corpuscular nature of the 'virus' of foot-and-mouth disease." This sort of uncertainty regarding the nature of the filtrable agents was to

continue for a long time. This was so in spite of the fact that many more diseases of plants, animals, and human beings were to be traced to such agents.

It seems rather surprising that it took another thirty five years before this controversy was finally laid to rest when Dr W.M. Stanley of the USA could obtain the agents responsible for causing mosaic of tobacco in a crystalline form. One reason for this delay could be that scientists during this period did not attempt chemical analyses of the sap. Presumably they were looking for submicroscopic particles even though there was no evidence whatsoever indicating their presence. Lack of powerful microscope was, of course, the main stumbling block. The days of electron microscope were yet to come.

DISEASES AND FILTRABLE AGENTS

The discovery of the existence of filtrable agents capable of causing infectious diseases opened up new channels in research. Scientists were quite puzzled about their characteristics. What bothered them more was the possibility that these agents might be responsible for causing many of the diseases about which they were uncertain. In those days and even earlier than that, several diseases of plants, animals and human beings were being investigated for their casual agents. Nobody was certain about them. After it was known that the mosaic of tobacco was caused by a new type of substances, scientists tried to find out if the other diseases were also similarly caused. They found, to their utmost relief, that most of these were indeed so.

One of the first diseases of human beings shown to be caused by filtrable agents was the dreaded yellow fever. At the beginning of the current century this was a serious problem with the authorities of the Central American countries like Panama. The disease often appeared in an epidemic form. To investigate into the disease thoroughly, the then Government of the United States had appointed a commission under Dr Walter Reed, a famous physician of the time. He and his associates, especially Dr Carlos Finley of Cuba, were able to successfully demonstrate in 1901 that this disease was caused by filtrable agents. They also were the first to establish that the filtrable agents were carried from infected to healthy persons through the agency of insects. Such living carriers of disease causing agents are called *Vectors*.

Rabies was another important human and animal disease whose causal agent was found to be filtrable in those early days. In 1903

one Dr Negri demonstrated that the nerve cells of rabies infected dogs contained prominent crystalline bodies. The presence of such inclusion bodies is a very important feature of the disease. Of the other diseases the causes of which were known early, poliomyelitis is one. In 1909, Dr Karl Landsteiner, the famous discoverer of human blood groups, and his colleagues demonstrated the filtrable nature of these agents.

In 1911 another important discovery opened up a new line of thinking in the study of viruses. In that year it was discovered by an American Virologist, Dr Peyton Rous, that *sarcoma* (a type of cancer) of chicken was caused by filtrable agents. Similar findings were also reported earlier by Drs Ellerman and Bang. However, their reports were not confirmed. A few other types of cancer in animals were similarly traced to filtrable agents. Scientists began to consider the possibility of cancer of human beings to be caused by such filtrable agents. The contribution of Dr Rous got a belated recognition and he was awarded the Nobel Prize some fiftyfive years later in 1966.

Causes of several plant diseases were also similarly traced to filtrable agents. In 1894, Dr Hashimoto and his colleagues in Japan demonstrated that the causal agent of the dwarf disease of rice was filtrable. They also noted that the disease was transmitted by leafhoppers, a type of insects. This was an important discovery but they could not connect the filtrable agents with these insects. The relevance of disease carriers was not understood by them. Its real significance was to be known later. During the first decade of the current century a few more plant diseases were shown to be caused by these entities. In this regard the contribution of Dr Allard in the United States of America in determining the cause of diseases of several plants belonging to the family Solanaceae, like potato and tomato, is worth mentioning.

The filtrable agents by this time had come to be known as viruses. Louis Pasteur and his contemporaries used the term loosely to refer to any unknown agent causing some disease or the other. With the discovery of the fact that some of these agents were able to pass through bacteria retaining filters, scientists began to differentiate between the two types that were automatically formed. One category could pass through bacterial filters while the other could not. It was not definitely known whether the latter were particles or not. However, the disease causing saps obtained from infected plants were thought to possess poisonous substances or 'viruses.' Hence this name came to be accepted in the scientific literature. The term soon became generally and universally recognised.

BACTERIOPHAGES

Just before the World War I another important discovery was in the making. In 1915, F.W. Twort, an English bacteriologist was investigating certain types of soil bacteria. In some of his experiments he had to use bacteria free soil extracts to culture the bacterial strains. Some of these bacteria, he found, were not growing at all. On the contrary, they seemed to have been completely destroyed. When he repeatedly observed the same phenomenon to occur, he concluded that these bacteria were destroyed by some filtrable agent present in the bacteria free soil extract.

In the year 1916, similar observations and the same conclusion were reported by a Canadian worker named d'Herelle, who too was working independently on similar problems at that time. He further demonstrated that only specific types of bacteria were destroyed in the presence of extracts obtained from certain specific soil samples. Other categories of bacteria remained unaffected. He also gave a new name to these bacteria destroying viruses. He called them *Bacteriophages* (Greek word *Phagein* means 'to eat') literally meaning eaters of bacteria. Scientists of that period, at least a few of them, became very hopeful about this discovery. They calculated that perhaps a possible antidote against disease causing bacteria has been discovered. This hope, alas, was later proved to be a false one.

Study of viruses had acquired momentum by then. More and more diseases were eventually demonstrated to be caused by them. Today, the number of viral diseases of plants, animals and human beings can be counted in hundreds. However, even when the existence of a large number of disease causing filtrable agents was known, surprisingly little information was available regarding their physico-chemical properties. For one thing, very little work had been done in this respect at that time. Lack of sophisticated and better equipments was one of the main reasons behind such apparent lack of interest. Another reason could be that scientists then were mainly thinking in terms of some bacteria like micro-organism. The idea of a different category of agents was most probably never seriously thought of. And quite understandably so.

TECHNOLOGICAL BREAKTHROUGH

By the end of the third decade of the present century, two major technological advancements had been made. These were the developments of the techniques of ultra-centrifugation and electron micro-

scopy, respectively (see the next chapter for details). The first enabled one to separate very small and light particles from a suspension. The other made it possible for one to see things which could not be seen with the help of even the most powerful of optical microscopes. The usefulness of these powerful techniques in the study of viruses became immediately clear. The controversy regarding the nature of viruses could now be solved once for all.

In 1933 Dr Max Schlesinger of the United States reported for the first time the isolation of a virus. He could successfully do so utilising the newly developed technique of ultra-centrifugation. The virus isolated was the bacteriophage *WLL*, infecting the bacterium *Escherichia coli*. He went further and analysed these isolated particles chemically and found them to contain proteins and phosphoric acid. He suggested the presence of nucleic acid in these particles. In one stroke he solved two problems. He proved that viruses were indeed particulate, and that these were composed of simple biochemical molecules. The idea, therefore, gained that viruses might be very simple organisms with only the essential biochemical substances.

CRYSTALLIZATION

Despite Schlesinger's impressive efforts, everybody was not convinced with his conclusions. They mainly doubted the purity of his preparations. They thought these were mixed up with the cell residues of the host bacterial cell which gave the relevant positive tests. All doubts were, however, set to rest in 1935 when an American chemist Dr W.M. Stanley was able to obtain a virus in its purest form. The virus thus obtained was an old puzzle which caused the mosaic disease of tobacco. In fact he was able to obtain the virus in its crystalline form. It was a most interesting and remarkable achievement. Stanley was eventually awarded the Nobel Prize for his contribution. In this context the pioneering contribution of two scientists Drs Vinson and Petres, who were the first to demonstrate, in 1931, the possibility of crystallising the infectious agent present in the sap obtained from leaves of mosaic diseased tobacco plants should also be remembered. Using such precipitating agents as acetone and alcohol, they could successfully demonstrate the precipitation of a material which shows all the infective properties of the original juice. They could not, however, successfully isolate a chemically pure crystalline entity from the sap, an achievement rightly credited to Dr Stanley.

Once a virus could be obtained in a pure crystalline form, further investigations could be possible. It was shown by Stanley that these

particles or crystals of tobacco mosaic virus (TMV) were actually aggregations of numerous small particles. Two British biochemists Drs N.W. Pirie and F.C. Bawden analysed these particles chemically and demonstrated that these were made up of proteins and ribonucleic acids (RNA). These two scientists were associated in a fruitful partnership for a number of years. During this period they brought out the chemical nature of several other viruses obtained mainly from plant sources. One important feature of viruses was soon known from these results i.e. viruses were invariably made up of proteins and nucleic acids, and that the nucleic acid present in a particular virus was either RNA or DNA (deoxyribonucleic acid). The distinguished contribution of these two scientists went a long way in helping us in understanding the chemical nature of viruses. Dr Pirie was later awarded the Nobel Prize for his achievements.

Although the chemistry of viruses was fairly well known by the forties of the current century, their physical nature was still not very clear. It is true that electron microscope had by then been discovered. However, the techniques needed for studying viruses with them were still to be perfected. Due to the continuous efforts of a number of scientists these problems were soon overcome. The studies by Drs Williams, Sidney Brenner and Anderson are worth mentioning in this respect. The morphology of many virus particles was soon established.

Meanwhile, other aspects of viruses were beginning to get attention. Dr Salvador Luria and Dr Max Delbruck in the United States of America and Professor Andre Iwoff in France, along with their colleagues, were initiating investigations into various aspects of their physiology. That viruses do multiply was quite obvious. Otherwise they would not be able to infect a large number of individuals starting from a single source. That they were subject to normal genetical alterations, like mutation, was also known from the ability of scientists to induce one type of virus, infecting a particular host to infect a new, hitherto resistant one. Therefore, these aspects were being studied with added emphasis.

During that decade some important viral diseases, like influenza and polio, were getting renewed attention. People soon realised that unless the disease causing viruses could be isolated and grown in the pure form in laboratory conditions; correct precautionary measures like vaccines, could not be developed. Efforts with this aim in view were being made. Success was achieved in 1949 when Dr John Enders in the United States was able to cultivate polio virus in laboratory. He utilised the newly developed tissue culture techniques for this purpose.

VIROLOGY COMES OF AGE

The next two decades saw a tremendous increase in the scope and dimension of virology. Ultrastructure of important viruses such as the TMV and the *T* series of bacteriophages were brought out. Several genetical aspects of the bacteriophages were established. These viruses were recognised to be very useful experimental tool. Methods for preventing and curing viral diseases were developed. Dr Jonas Salk and Dr Albert Sabin developed vaccine against polio thereby enabling millions of children to be saved from this dreadful disease. New types of viruses were also discovered. People started actively considering the artificial synthesis of viruses. Perhaps, they thought they could synthesise life in this manner and understand the phenomenon better.

The science of virology has travelled a long way since the days of its inception. Some idea as to how it progressed may be obtained from the information provided in Table 1.1. However, many unsolved problems and questions still remain to be answered. In fact, more questions have arisen out of the knowledge that has been gathered to solve the earlier problems. One of the most fundamental of these questions relates to their biological nature; we are not sure if they are living or for that matter non-living. Because they exhibit the characteristics of both.

TABLE 1.1. IMPORTANT EVENTS IN THE DEVELOPMENT OF VIROLOGY

| Year | Event | Author(s) |
|------|---|----------------------------|
| 1796 | First successful vaccination | Edward Jenner |
| 1880 | Discovery of rabies as an infectious disease; possibly caused by submicroscopic agents other than bacteria | Louis Pasteur |
| 1886 | The mosaic disease of tobacco was proved to be infectious; probably caused by agents other than bacteria | Adolf Mayer |
| 1892 | Tobacco mosaic disease was shown to be caused by entities that could pass through filters capable of retaining bacteria | Dimitry Iwanowski |
| 1896 | Emergence of the concept of <i>Contagium Vivum Fluidum</i> | M.W. Beijerinck |
| 1898 | Foot-and-mouth disease of cattle traces to filtrable agents | F. Loeffler and P. Frosch |
| 1901 | Yellow fever shown to be caused by filtrable agents | Walter Reed; Carlos Finley |

| <i>Year</i> | <i>Event</i> | <i>Author(s)</i> |
|-------------|---|---|
| 1911 | Discovery and confirmation of cancer producing capacity of filtrable agents in chicken and fowl. | J. Ellermann and B. Bang; Peyton Rous |
| 1915 | Bacterial destruction by filtrable agents reported | F.W. Twort |
| 1916 | Specific infection of bacteria by specific filtrable agents demonstrated. The term bacteriophage is coined. | F. de Herelle |
| 1925 | First successful cultivation of a virus (vaccinia) in tissue culture | F. Parker and R.N. Nye |
| 1933 | First successful isolation of a virus, the bacteriophage WLI | M. Schlesinger |
| 1933 | Light microscopic observation of the filtrable sap obtained from infected leaf of tobacco | W.N. Takahasi and T.E. Rawlins |
| 1935 | A virus could be isolated in a crystalline form for the first time. The virus was TMV obtained from saps of infected tobacco plants | W.M. Stanley |
| 1935-38 | Chemical nature of TMV and many other viruses determined | N.W. Pirie and F.C. Bawden |
| 1939 | Discovery of mutation in viruses | M. Delbruck; C.A. Knight |
| 1941-46 | Development and improvement in electron microscopy of viruses | R.C. Williams and R.W.G. Wycoff; S. Brenner and R.W. Horne; T.A. Anderson |
| 1942-48 | Elaboration of the process of replication in bacteriophages | S. Luria and M. Delbruck; A. Lwoff |
| 1949 | First successful cultivation of a virus (polio) in human tissue culture | J. Enders |
| 1952 | Discovery of transduction | J. Lederberg and N. Zinder |
| 1952 | Discovery of proteins and nucleic acid to be the non-infective and infective parts of a bacteriophage respectively | A. Hershey and M. Chase |
| 1953 | Discovery of temperate phages | A. Lwoff and E. Wollman |
| 1956 | Demonstration of reconstitution of tobacco mosaic virus | W.H. Takahasi; H. Frankel-conrat |
| 1957 | Discovery of interferons | A. Issacs and J. Lindemann |
| 1955-57 | Elucidation of the ultrastructure of TMV. | R. Franklin, A. Klug and K.C. Holmes; C.A. Knight and J. I. Harris; A. Gierer |

| Year | Event | Author(s) |
|---------|---|---|
| 1957 | Successful vaccination against polio. Development of oral vaccines. | J. Salk and A. Sabin |
| 1959 | Discovery of $\phi \times 174$, the bacteriophage with single stranded DNA | R.L. Sinsheimer |
| 1959 | Elucidation of the ultrastructure of the bacteriophages T ₂ | S. Brenner, G. Strisinger and R.W. Horne; D. Crowther |
| 1960-65 | Intensification of studies on inhibition of viral multiplication. Development of chemotherapeutic agents | I. Tamm, H. Eggers; R.R. Wagner; H.E. Kaufmann; D. J. Bauer <i>et al.</i> |
| 1963 | Discovery of Cyanophages | W.K. Zoklick <i>et al.</i> R.S. Shafferman and M.E. Moris |
| 1964-70 | Discovery of RNA dependent DNA synthesis (reverse transcription). A unique phenomenon noted in viruses alone. | H. Temin and D. Baltimore |
| 1966 | Formulation of a unified system of classification of viruses | A. Lwoff, R.W. Horne and P. Tournier |
| 1967-72 | Attempts towards artificial synthesis of viruses like the TMV and the bacteriophage $\phi \times 174$ | A. Kornberg and M. Goulian; R.L. Sinsheimer; G.W. Cochran |
| 1971 | Discovery of the occurrence of transduction in human fibroblast cells. | C.R. Merrill, M.R. Ceier and I.C. Petricciani |
| 1973 | Discovery of virus caused cancer in primates. | D. Schidolovski and R. Ahmad, J. Gallow |
| 1973-80 | Emergence of indications that Herpes Simplex virus may cause human cancer. | A. Sabin and E. Tam; F. Dress |
| 1976-77 | Genetic mapping of the bacteriophage $\phi \times 174$ | F. Sanger |
| 1977 | Emergence of new viral diseases | Pattyn |
| 1979 | Elucidation of nucleotide sequence of Hepatitis B virus genome | Gailbert |
| 1979 | Cloning of Hepatitis B virus genome in <i>E. Coli</i> genome | Sninsky |

Some virologists have come to regard them as something unique, something different; something which could not be placed within the general framework of either the animate or the inanimate world.

Another question-mark relating to hitherto unknown viral diseases is coming to notice, signalling ominous forebodings. Outbreaks of epidemics of haemorrhagic fever in Sudan, as recently as in 1975 and 1976, have been traced to viruses (Pattyn *et al.* 1977). One can only hope that such are but a few diseases.

Study of viruses has now come to occupy an important position as a major branch of the sciences. The problems posed by this are not complex and puzzling, they are also extremely interesting. You can have an idea about their attraction from the fact that persons trained in different branches of the sciences have decided to devote themselves to the study of viruses. Nuclear physicists and botanists, biochemists and entomologists are often found rubbing their shoulders working on the same problem.

What we know about viruses, though substantial, offers us only a partial view of these mysterious agents. Yet, what it offers is immensely fascinating. And equally intriguing at the same time. Researches carried out in different parts of the world during the next few decades are expected to open up new vistas. Views of a twilight zone within and yet beyond the living world is now available. One hopes the scene to gradually clarify in the foreseeable future.

Summary

Virology, the discipline dealing with viruses, is of comparatively recent origin; it is roughly a century old. However, several incidences linked directly to viruses have been known to mankind from the very dawn of civilization; as for example, such diseases as small-pox, rabies or the mosaic disease of tobacco or such incidences as "tulip break." In fact, that most outstanding example of applied virology, vaccination, introduced by Jenner in 1776, antedates the actual discovery of viruses by almost one hundred years.

Credit for discovery of viruses goes to Iwanowski (1892). However, contribution of pioneers like Pasteur, Mayer and Beijerinck can never be overemphasized. Iwanowski demonstrated that the agents responsible for causing the mosaic disease of tobacco were 'filtrable' indicating the existence of disease causing agents smaller than bacteria. Their distinct nature was established later by Beijerinck who described them as 'contagium vivum fluidum' or 'living infection fluid.'

The 'filtrable' nature of the causal agents of many hitherto unestablished diseases, both plant and animal were gradually discovered. 'Filtrable' agents destroying bacteria was discovered too. Moreover, it also became clear that these mysterious agents were always to be found in association with some living organism or other but never alone. The term virus gradually became associated with these 'filtrable' agents.

The real breakthrough in the progress of virology was achieved in the forties with the development and utilisation of sophisticated techniques like electron microscopy, X-ray crystallography and ultracentrifugation. At about the same time another development went a long

way in establishing the true physico-chemical and biological nature of viruses. This was the successful crystallization of tobacco mosaic virus (TMV) particles by Stanley (1935).

Utilizing these techniques the information concerning the chemistry and physics of viruses was obtained at a rapid rate. New viruses were discovered. Ultrastructure of well known viruses like TMV and bacteriophages were elucidated. Viruses could be successfully cultivated in the laboratory thus rendering the task of getting viruses in the pure form comparatively easier.

Several aspects of the physiology of viruses, particularly their ability to reconstitute and replicate were also established. That they could replicate only at the expense of the metabolites and energy of the host, while guiding the entire operation through their own genomes, added an extra dimension to their characteristic.

A duality in their character, involving traits, purely inanimate and also purely animate came to be recognised. Their capacity to mutate and recombine genetically was established. Their unique biology was accepted. All this happening during the course of last few decades.

In recent years the emphasis has been on the proper assessment of the role played by viruses in causing diseases and to develop ways and means to fight them. With the establishment of a viral connection to many a disease, particularly of animals, proper immunization procedures have been developed; chemotherapeutic agents have been discovered and prepared. Control of viral diseases by checking their modes of transmission has been achieved. The role viruses play in causing cancer in mammals and their organisms is being investigated extensively and intensively all over the world.

Though young, the science of virology has already assumed a significance which is second to none. Many ideas concerning them are getting clarified. One hopes the future will bring in a better understanding and a better adaptation to these mysterious agents which defy full understanding as yet.

Chapter Two

GENERAL TECHNIQUES IN VIROLOGY

Once the existence of '*filtrable agents*' or viruses was established, questions as to what these viruses were, and what was indeed their physico-chemical nature, became crucial. Scientists started probing into these questions from the very early days of Virology. In the beginning, the information that could be obtained by the application of comparatively simple techniques of those days were insufficient. The information could give rise to some incomplete and vague ideas only. People were more or less moving in the dark. With the development and use of better instruments and techniques, like the electron microscopy and X-ray crystallography, the situation gradually changed and new methodology came to be applied. More and more information was gained. Old ideas soon became outdated to be gradually replaced by newer and better ones. The fundamental questions were getting to be solved, one by one.

The early ideas about viruses were based upon incomplete knowledge about a few of their characteristics. For instance, it was thought that they were invisible; nobody could ever see them. They were supposed to be filtrable, *i.e.*, they could pass through filters which could keep bacteria back; and it was assumed that they could not be grown in the laboratory like one could easily grow bacteria. All these ideas gained acceptance, at least for some time, because the techniques available in those days could not help the scientists know anything in more detail.

In due course electron microscopes were developed and used, filters with very fine pore became available, and it was discovered that cultivation of viruses in the laboratory was possible. The situation thus changed. Scientists realized that viruses were indeed visible; not only that, these were cultivable and could be retained or separated with the help of better filters. In this manner new and radically opposite ideas developed, replacing the old ones.

Some of the very basic questions regarding the nature of viruses were thus solved. But the answers themselves brought forward many more questions. Deeper our knowledge went about viruses, even deeper went our ignorance about them. Virologists all over the world have

been and are busy in investigating numerous aspects of viruses today. Uncertainties about them are gradually being removed. New informations and newer concepts are emerging. More are sure to emerge in the future enabling us to have a more harmonious and complete picture of these unique particles.

We have indicated how development and successful utilisation of improved techniques have played a very important role in improving our knowledge regarding viruses. Therefore, we shall first consider the various important techniques that have been and are being used by scientists to study viruses before going into what is known about these mysterious particles.

The general techniques that have been developed and employed from time to time to study viruses could be categorized under four main heads. These relate to cultivation, isolation, characterization and enumeration of viruses, respectively.

CULTIVATION OF VIRUSES

A unique feature associated with viruses is that they are never found alone. They are noted without fail, in the presence of or in association with some living cell or the other. In fact they are totally dependent upon their host cells for their very existence. Their total host dependence makes it extremely difficult for one to study viruses; chances of getting good insight into them in natural condition, *i.e.* within the host cell, are very limited because the internal characteristics of the host cell are likely to interfere with the observations. For all these reasons it has been found desirable that viruses are cultivated or grown in the laboratory itself. *First*, this would enable one to get sufficient amounts of the virus particles at any given time. *Secondly*, this would mean lesser degree of contamination from the host cell material, ensuring a purer virus sample to be studied later on.

Viruses cannot be cultivated like any other micro-organism, as for example, the bacteria. Since viruses are host dependent totally, it is not possible to cultivate them solely in the presence of organic or inorganic nutrient medium. They can be grown only if living cells and tissues are used as the culture medium. These tissues and cells would act as the host for the virus in the laboratory conditions.

For this purpose, the relevant cells or tissues must be cultivated first. Thus, for growing viruses infecting bacteria, bacterial cultures will have to be prepared. Similarly, cultures of tissues or cells of higher plants and animals will have to be prepared before attempting the cultivation of plant and animal viruses. The necessity of growing

specific host cells for cultivating a specific type of plant or animal virus has also to be remembered.

Cultivation of bacteria of the desired type is relatively easy. Standard procedures and culture media are easily available. However, the cultivation of tissues or cells of plants and animals is a more complicated affair. The entire process involving such cultivation is commonly referred to as *tissue culture* or *cell culture*. In the former pieces of tissues are used; in the latter dispersed cells of the tissues or loose cells. All the steps in the operation of the procedure have to be carried out in conditions that are aseptic (free of germs). Care has to be taken to prevent infiltration and growth of unwanted organism like bacteria and fungi. Usually antibiotics such as penicillin and streptomycin, are added to the culture medium to prevent the possible development of contaminating micro-organisms.

Cultivation of Animal Virus

Cultivation of the proper virus type necessitates the cultivation of proper tissue or cell culture. In certain cases organized tissue or even live animals are used.

Cultivation of Animal Cells

Supposing we wish to culture cells of a particular type of animal tissue. To do this, the tissue is first removed from the organism concerned. This organism is usually a suitable host for the virus that is to be later cultivated. The tissue is then broken down into its constituent cells by utilizing suitable physical means. Homogenization in a homogenizer is a common procedure that is utilized. The complete tissue is thus converted into many small pieces.

The next step would be to convert these pieces into their constituent cells. This step is known as dispersion of the cells from the tissue. This is usually done by breaking down the cementing material joining the cells with the help of enzymes. Since these cementing materials are mostly proteinaceous, proteolytic (protein digesting) enzymes like *trypsin* are usually used for the purpose. This step is, therefore, referred to as Trypsinization.

Before trypsinization is actually started, tissue fragments are washed with salt solutions. Sterile physiological saline or other types of solutions (like Hanks' solution or Earle's solution) may be used.

The chemical compositions of two of the washing solutions generally used are as follows:

Hanks Balanced Salt Solution

| <i>Solution I (g/litre)</i> | | <i>Solution II (g/litre)</i> | | <i>Solution III</i> | |
|-----------------------------|-------|--|---------------|---------------------|---------------|
| NaCl | 160 g | NaHPO ₄ 12 H ₂ O | 3.04 g | NaHCO ₃ | 1.4 per cent |
| KCl | 8 g | KH ₂ O ₄ | 1.2 g | Phenol Red | 2.04 per cent |
| MgSO ₄ | | | | | |
| 7H ₂ O | 4 g | Glucose | 20.00 g | | |
| CaCl ₂ | 2.8 g | Phenol Red | 0.04 per cent | | |

One part of solution I and one part of solution II slowly combined added to 18 parts distilled water 5ml of solution III added to the mixture of solution I & II before use.

Earle's Balanced Salt Solution

| <i>Solution A (g/litre)</i> | | <i>Solution B (g/litre)</i> | |
|----------------------------------|------|-------------------------------------|-----|
| NaCl | 68.0 | CaCl ₂ | 4.0 |
| KCl | 4.0 | MgSO ₄ 7H ₂ O | 2.0 |
| NaH ₂ PO ₄ | 1.25 | | |
| NaHCO ₃ | 22.0 | | |
| Glucose | 10.0 | | |

To prepare the washing medium, 10 parts of solution (A) and five parts of solution (B) are to be mixed with 85 parts of distilled water.

The washed tissue fragments are then placed in a flask with sterile trypsin solution at 4°C about 18 hours. During this period, the tissue fragments are gradually dispersed into their cellular components. The cells float in a suspension and for all practical purposes are comparable to micro-organisms. Presence of chemicals like ethylene-diamine-tetra acetic acid (EDTA) helps in the dispersion of the cells. The cells may then be further centrifuged and resuspended in a suitable washing medium. Such centrifugation and washing should be repeatedly done.

The washed suspended cells may then be cultivated in a suitable growth medium. A typical medium for animal tissues and cells has the following composition:

| <i>Ingredients</i> | <i>Parts per cent (in volume)</i> |
|-----------------------------------|-----------------------------------|
| Selected Bovine Serum | 5 |
| Lactalbumin hydrolysate | 10 |
| Hanks' BSS | 82 |
| NaHCO ₃ (2.8 per cent) | 2 |
| Antibiotic mixture | 1 |

Cultivation is done after adjustment of the number of cells per unit volume. The required number of cells is suspended in the growth medium taken in tubes or flat bottom flasks. The entire culture is then incubated at 36°C for 72 hours.

When grown under proper conditions, the cells in culture multiply and cover the bottom of the glass container with a thin but continuous

layer, which is often one cell thick. Such cell layers are called *monolayers* (Fig. 2.1). Development of uniform monolayer cultures proved to be a major advantage in the cultivation of viruses. This technique was further improved upon by Dr Dulbecco and his associates in the USA.

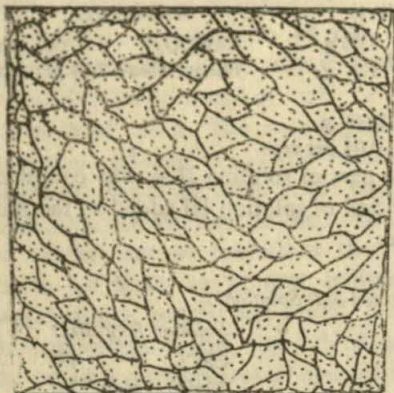


FIG. 2.1. Diagram of animal tissue cells in monolayer culture. The cells were originally dispersed from a suitable tissue, like kidney, lungs or cancerous growths utilizing various physical and chemical means. The cells are settled in an uniform layer at the bottom of the container. Note the contiguous outline of the cells.

They found that the monolayers could be developed on agar containing the necessary nutrients. Virus particles grown on such monolayers are extremely uniform in growth. In certain cases, the dispersed cells are not allowed to settle down at the bottom of the container. Rather they are kept floating by shaking the flasks continuously on a mechanical

shaker. The cells remain suspended and, therefore, this type of cultures are called *Suspension Cultures*.

A culture derived directly from a tissue is called the *Primary Culture*. Subsequent cultures developed from the primary cultures are called the *Diploid Cell Strains*. Sometimes it so happens that the diploid cell strains die off. In some other cases they live indefinitely in culture but in a slightly modified form. Such cell strains are known as *Permanent Cell Lines*. Permanent cell lines derived from a single separated cell are called *Clones*. One common example of such clones is the well known Hela cell strain derived from cancerous human cervical tissue.

Cells of various types of tissues of animals may be cultivated. But more commonly, fibroblast and muscle epithelial cells are used for the propagation of viruses.

A vigorously growing monolayer or suspension culture is then inoculated with the type of virus to which it is susceptible. With routine work, the host specificity of the virus is predetermined. However, while dealing with uncertain virus types, the proper kind of tissue is selected after trial and error. The inoculation is done by mixing or spreading the viral suspension with the cultivated host cells. The virus particles infect the host cells in due course. They multiply in number

within the host cell and eventually come out by destroying the host cell. They are thus liberated into the surrounding medium and in fact the neighbouring cells. The cell cultures soon look disintegrated. In case of monolayer cultures of tissue cells the spread of virus infection is very rapid due to the uniform growth of the host cells. For this reason, the growth of the virus too is very rapid.

The initially formed virus particles soon lead to the production of many more viruses. The initial infection of a cell by a virus particle occurs at random. However, the infections of the other cells by the newly formed virus particles are restricted to certain areas near the initially infected cells. Soon, these areas appear to be completely disintegrated and take the shape of white patches against the background of the host cells. These patches are called *Plaques*. The appearance and form of such plaques are virus specific. Therefore, these act as indication of the characteristics of a virus (Plate I).

Cultivation of Viruses in Organised Tissue

Prior to the development of suitable tissue culture techniques, virologists often used organised tissue structures for virus cultivation. Such animal tissues as the chorioallantoic membrane of chick embryo are commonly used (Woodruff and Goodpasture 1931). Although their use is not as widely prevalent as earlier, organised tissue structures are still considered helpful in culturing certain virus types (Plate II).

Cultivation of viruses in organised tissues like chick embryo necessitates a different type of approach. These are live embryos so very beautifully packed in their shells. For all practical purposes, these themselves behave as tissue cultures (Fig. 2.2).

In order to cultivate viruses on these, the procedure adopted is very simple. The egg containing the embryo usually has an air space at the large end. The position of this sac is first determined. The shell over the air sac is then cut off and removed. The membrane adjacent to the shell is then pierced. The inner chorioallantoic membrane is thus exposed. At this stage, the embryonic fluid may ooze out. The shell membrane is then removed. The rest of the embryo gets exposed and is ready for use. Virus suspension to be cultivated is taken in dropper and gently spread over the exposed embryo. After inoculation is thus completed, the open area of the shell is sealed with a cellophane membrane. The sealed eggs are then incubated for several hours as in hatching. The virus particles infect the membrane at random and create pock marked appearances against the translucent background. These indicate viral decisions.

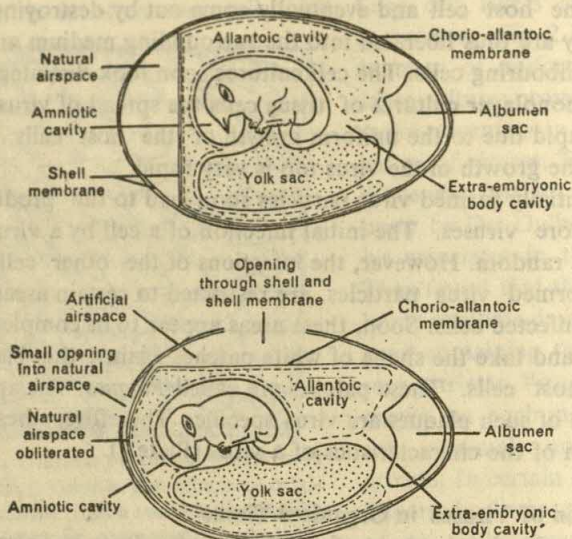


FIG. 2.2. The membranes and cavities of the embryonated hen's egg. Viruses may be grown in the cells lining the allantoic cavity, the amniotic cavity, or the yolk-sac, or in the cells on the upper surface of the chorio-allantois. In the later case, the chorio-allantoic membrane is usually 'dropped' artificially, as in the lower picture.

Live Animals as Laboratory Virus Reservoirs

Sometimes, natural hosts of certain animal viruses are used and maintained as stock reservoirs. This has certain advantages over cultivation of viruses. For instance, the complicated procedures for cultivation of tissues are dispensed with. Also the fear of contamination is no more there. However, expenses could be great, particularly if larger animals are used. Besides, sometimes chances of getting infection while cultivating disease causing viruses may be there. For instance, Herpes B Virus can be cultivated in monkeys but can be dangerous for human beings as it causes encephalitis.

In recent years, use of small animals like mice and guineapigs has been quite extensive, particularly the former. Maintenance of strains of murine leukoemia virus (MLV) in selected mice strains is an illuminating example (Gross 1953).

Cultivation of Plant Viruses

Dr P.R. White (1934) was the first to grow plant viruses in tissue culture. He could successfully cultivate tobacco mosaic virus in excised

tomato root tips.

The method he developed was simple. It has since been widely practised by plant virologists all over the world. In this method, a systematically virus infected tomato plant was used. Its stem was cut up into segments which were washed thoroughly and were suspended by threads in conical flasks containing little water. Care was taken to see that the segments touched neither the water nor the side walls. The segments were allowed to develop roots. After a period of time, the root tips were removed and placed in flasks containing 50 ml of a suitable nutrient medium.

Chemical composition of one such medium is as follows:

| | |
|---|-----------------|
| Ca(NO ₃) ₂ | 0.60 millimols |
| MgSO ₄ | 0.30 millimols |
| KNO ₃ | 0.80 millimols |
| KCl | 0.87 millimols |
| KH ₂ PO ₄ | 0.09 millimols |
| Fe ₂ (SO ₄) ₃ | 0.006 millimols |
| Surase | 2 per cent |
| Yeast and extract | 0.01 per cent |

The tips were allowed to grow for about a week. Afterwards, the surviving cultures were cut into uniform length of about 1 cm. These were then subcultured in fresh medium as before. The root tips of the subcultures were removed and one or two of these were selected and stocked for further subculturing. Viruses were found to be multiplying in these tips and could be used for studies taken up subsequently.

Using the above technique it has been possible to culture such viruses as tobacco necrosis virus and wound tumour virus. The degree of success achieved with different viruses has been variable. It has been found that the type of virus, the nature of the host and nutritional factors considerably affect the growth of viruses.

Plant viruses are normally cultivated on tissues obtained from already and systemetically infected plants. This is because it has been found to be difficult to infect healthy plant tissue cultures freshly with a virus.

Nevertheless, the need for *de novo* culturing of viruses sometimes arises. In such cases healthy tissues, not necessarily of the natural host, are first cultured. Later, it is infected artificially by the virus to be grown. Methods to do so have been developed and perfected in recent years. In some cases the viral inoculum is directly poured on the growing tissue. To make the infection steady and quick, the tissue surface is pricked with the needle or rubbed with abrasive quartz or carborandum powder. Sometimes, the natural vector of a disease, which itself harbours the virus particles, is used to inject the cultured

tissues (Kassanis *et al.* 1958; Chill *et al.* 1966).

A more recent development has been the use of isolated plant protoplasts for culturing plant viruses. (Sarkar 1976) This method has the additional advantage in that protoplasts can be uniformly and synchronously cultivated.

ISOLATION AND PURIFICATION OF VIRUSES

We have seen how viruses are cultivated. However, in order to study them thoroughly one must have them free from unnecessary contaminating material, such as components of the tissue cells. It is essential that the viruses are available in a reasonably pure form. To achieve this they must be isolated first and then suitably purified. The principal techniques used for this purpose are (a) ultra-filtration; (b) ultra-centrifugation; and (c) chemical precipitation. In recent years chromatographic, electrophoretic and solvent extraction techniques have also been increasingly used.

Certain points should be given the proper attention before any of the steps for isolation and purification can be taken up. These concern the availability of virus particles in active, infective form in the initial extract. Viruses, which are totally host dependent, remain, while inside a cell, sort of insulated against the vagaries of changing external temperature and pH conditions. Therefore, firstly, the operations for isolation have to be carried out at extremely low and constant temperatures. Thus adverse effects of temperature changes can be minimized, if not completely removed.

Secondly, the selection of proper pH condition of the extracting medium is important. Suitable buffers have to be used. Both inorganic and organic buffers are in use. With experience the choice of buffer for a particular type of virus is standardized to ensure minimal loss.

Ultrafiltration

This operation is based upon the fact that, generally speaking, virus particles have a smaller size dimension than any other micro-organism. Therefore, to separate viruses from amongst a mixture of variously sized particles, one has simply to use a filtration device capable of retaining the larger particles while allowing the smaller virus particles to pass through. For instance, let us suppose a viral suspension present in a bacterial medium is to be isolated by filtration. All one has to do is to allow the fluid suspension to pass through a suitable filter capable of allowing only the virus particles to pass

through, while keeping back the larger particles. Seitz type filters or porcelain G-5 filters may be conveniently used. The clear filtrate thus obtained should contain solely viruses.

In order to retain these virus particles the viral suspension may further be passed through ultrafilters made of cellulose acetate or collodion membranes (Elford 1939). The very small pore size of these filters enables them to retain the comparatively larger particles of the virus. Such membrane filters with graded porosity, like Millipore filters (manufactured by the Millipore Filters Corporation, USA) have accurately determined pore size. These can, therefore, be used to segregate particles having different sizes. Particles larger than a particular pore size get wedged in the small pores of these filters. Thus the smaller ones get through but may again be retained by filters having still smaller pore size. The filtered particles of a particular size can be brought in fluid suspension by washing the filters in reverse direction (Fig. 2.3). In recent years, diatomaceous earth has been increasing in use as a filtering aid. Charcoal and bentonite have

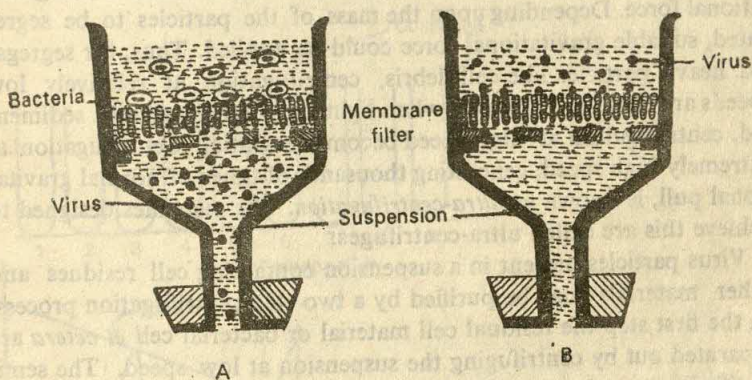


FIG. 2.3. Cross-sections of the filters capable of retaining bacteria (A) and viruses (B). Both of these are membrane filters A suspension carrying bacteria and bacterial viruses can be freed of both by successively passing it through A and then B. It is clear that the diagram drawn is not to proportion. Bacteria and viruses have been shown to be comparatively much larger than the filter for the sake of clear understanding.

also been successfully used. More sophisticated gel filtration techniques using agar-agar or 'sephadex' allow selective filtration of virus particles.

The filtration of viruses, no matter how small be the pore size, is not fool-proof against possible presence of other organisms. This is because there are a few other types of micro-organisms which have

size ranges comparable to that of larger viruses. The Rickettsiae, a pathogenic group of micro-organisms and mycoplasmatales, a type of bacteria are two examples of such organisms. Therefore, the technique of ultrafiltration is likely to be useless in case the viral suspension contains these or similar micro-organisms as well.

Ultra-Centrifugation

Out of the particles in a suspension the heavier ones have a tendency to settle down first. This is because of the attraction of gravitational forces. Usually, the process would be a slow one. In case of extremely small particles, the process of settling down or *sedimentation* may take an almost indefinite period. The rate of sedimentation could be increased by increasing the gravitational pull itself. This can be done by applying centrifugal force on the suspension. This is commonly achieved by rotating centrifuge tube containing the suspension in a centrifuge machine. Such rotation or centrifugation at a very high speed produces forces equivalent of many thousand times of the gravitational force. Depending upon the mass of the particles to be segregated, suitable gravitational force could be applied. Thus, for segregated heavy particles like cell debris, centrifugation at relatively low speeds are enough. However, when lighter particles are to be sedimented, centrifugation at high speed becomes essential. Centrifugation at extremely high speed, generating thousands of times of normal gravitational pull, is known as *ultra-centrifugation*. The machines designed to achieve this are called ultra-centrifuges.

Virus particles present in a suspension containing cell residues and other materials may be purified by a two step centrifugation process. In the first step the residual cell material or bacterial cell *et cetera* are separated out by centrifuging the suspension at low speed. The semi-purified suspension could be further purified by filtration. The filtrate containing the virus particles may then be subjected to ultra-centrifugation. The virus particles sediment and form a small heap or pellet at the bottom. This technique is called differential centrifugation. In this manner Schlesinger in 1933 was able to isolate the bacteriophage WLL.

In recent years, the technique of ultra-centrifugation has been modified to ensure greater resolution. Two commonly used modified techniques are known as *rate-zonal centrifugation* and *equilibrium density gradient centrifugation* (Vinograd 1959). In these methods, the viral suspension is mixed with appropriate gradient concentrations of sucrose and on caesium chloride in sodium chloride solution. The gradient is previously prepared by successively pouring equal volumes of

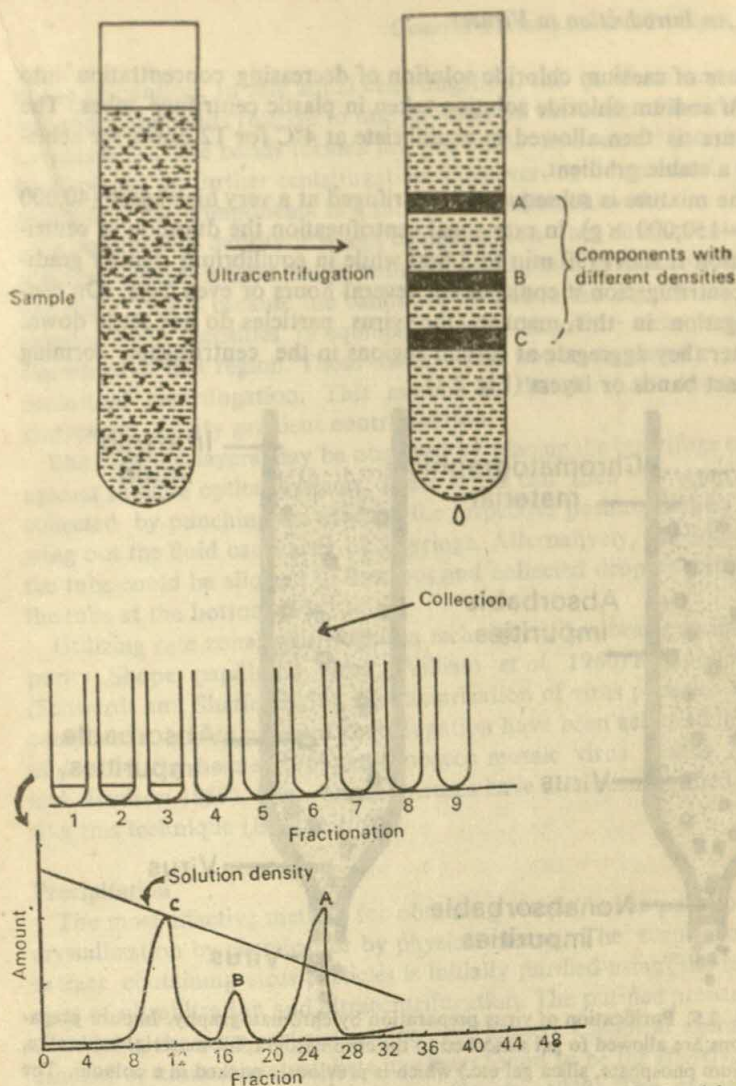


FIG. 2.4. Ultra-centrifugation (Equilibrium density gradient). Particles of different mass size present in the same medium can be segregated by this method. The medium is mixed with an appropriate concentration of sucrose or casium chloride solution and then centrifuged at very high speed (for instance, $40,000\times g$) for several hours. Different categories of particles separate out according to their densities and aggregate in layers at different regions (A,B,C). The heaviest particles from the lowermost layer and vice-versa. The individual categories of particles may be obtained separately by puncturing the tube at the bottom and then collecting the outflowing liquid in test tubes taking a small volume at a time. Generally, the major portion of a particular layer gets collected in one or two successive tubes. Here in the graph you will note that the layer C flows out first and is collected initially. Presence of separated particles in the tubes can be detected by spectrophotometric means. Usually macromolecules like proteins and nucleic acids, viruses are purified by this method.

sucrose of caesium chloride solution of decreasing concentration into .14 *M* sodium chloride solution taken in plastic centrifuge tubes. The mixture is then allowed to equilibrate at 4°C for 12 hours for achieving a stable gradient.

The mixture is subsequently centrifuged at a very high speed (40,000 $\times g$ —150,000 $\times g$). In rate zonal centrifugation the duration of centrifugation is short (30 min to 3 hrs) while in equilibrium density gradient centrifugation it could be for several hours or even days. On centrifugation in this manner, the virus particles do not settle down. Rather they aggregate at certain regions in the centrifugate, forming distinct bands or layers (Fig 2.4).

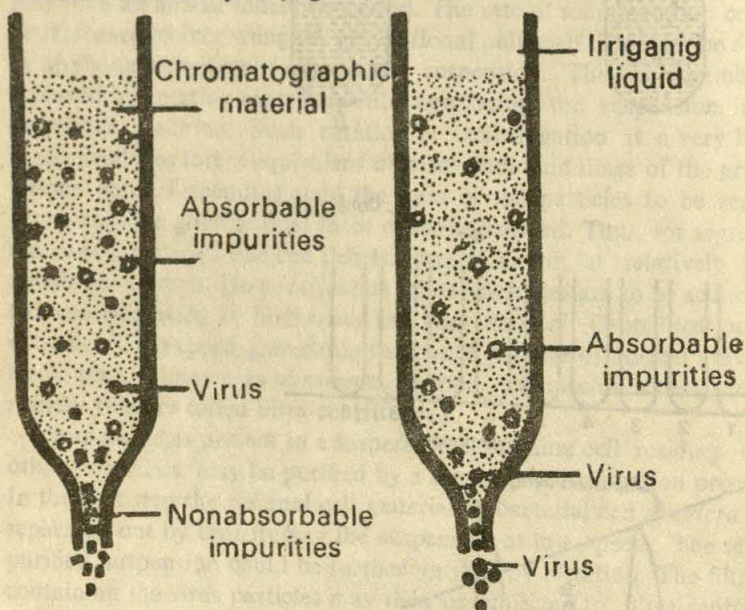


FIG. 2.5. Purification of virus preparation by chromatography. Impure preparations are allowed to get adsorbed to the chromatographic material (amberlite, calcium phosphate, silica gel etc.) which is previously packed in a column. The suspending fluid containing the preparation drains out Non-adsorbable impurities pass out along with it. Viruses are absorbed to the column material. So are adsorbable impurities. The column is later washed with large volumes of an appropriate salt solution ($MgCl_2$, $NaCl$, phosphate buffer). Viruses are preferentially washed out or eluted and hence come out with the washing solution. This is because they have more affinity towards the washing solution than do the adsorbed impurities. The latter remain adsorbed to the column. The preparations is thus freed of both the types of impurities.

The number of such bands indicates the number of types of particle

in the suspension. In rate zonal centrifugation, the particles separate out and form bands relative to one another, on the basis of their relative densities. The bands formed are not stable and would normally be displaced on further centrifugation. However, by this method, segregation of the components in a mixture is easily achieved.

In equilibrium density gradient centrifugation the components of identical densities form bands with stabilized position in the centrifuged fluid. This is because the bands are formed in the regions of the solution with densities in equilibrium with that of the virus particles assembled at that region. These bands would not be displaced even on prolonged centrifugation. This method is, therefore, also known as *isopycnotic* density gradient centrifugation.

The different layers may be observed by placing the centrifuge tubes against suitable optical systems. These layers can then be separately collected by punching the tubes at the respective position and by drawing out the fluid cautiously by a syringe. Alternatively, the liquid in the tube could be allowed to flow out and collected drop by drop by punching the tube at the bottom (Fig. 2.5).

Utilizing rate zonal-centrifugation technique it has been possible to purify Shope papilloma virus (William *et al.* 1960) and poliovirus (Schwerdt and Shaffer 1956). Characterization of virus particles using equilibrium density gradient centrifugation have been achieved in case of $\phi \times 174$ (Sinsheimer 1959) and tobacco mosaic virus strains (Sigel and Hudson 1959). Some animal viruses have also been purified utilizing this technique (Brakke 1960).

Precipitation

The most effective method for obtaining viruses in the pure form is crystallization by chemical or by physical means. The suspension or extract containing virus particles is initially purified using the techniques of ultrafiltration and ultracentrifugation. The purified preparation containing the virus particles is then precipitated. Chemical agents like ethanol or ammonium sulphate has been found to be the effective concentration for precipitating viruses (Stanley 1938). The precipitates obtained once could be redissolved in some suitable buffer solution and reprecipitated. In this manner more and more purified preparations are made available. Methanol, protamine and even yeast nucleic acid have all been found to be effective precipitants of animal viruses.

Precipitation can also be achieved by adjusting the hydrogen ion concentration (pH) of the suspension. Proteins, as you know, are ampholytes *i.e.*, they possess two types of surface charges, positive and negative, on them. Proteins remain the solution till the charges

are unequal in number and till they are all neutralized. Once that happens they precipitate out. For a particular type of protein only one pH level is capable of precipitating that type of protein. This pH is called the *isoelectric point*. pH can be adjusted by adding more cation (Mg^{2+} , Ca^{2+} , H^{+} etc.) and anions (Cl^{-} , PO_4^{3-}) in the form of salt solution. These ions neutralize the negative and positive charges respectively and the isoelectric point is reached. Thus proteins are precipitated out.

Since viruses have an outer protein coat, they can also be precipitated in this manner. Alfalfa mosaic virus is one of the first viruses to be precipitated by this method (Lauffer & Ross 1940).

Adsorption Chromatography

Apart from these three major techniques, a few more effective physico-chemical methods have been developed and used in recent years. One of these is adsorption chromatography. This is dependent upon the surface properties of the protein coats of the virus particles. Because of these the virus can get associated with or adsorbed on the surface of a solid but porous substances having similar properties (charge, etc.) on them. Substances like calcium or aluminium phosphate are generally used. These are packed on columns of glass tube designed specially to hold them. The packing of the chromatographic material has to be done under special conditions (Fig. 2.6.)

The viral suspension along with impurities is then poured on the column. The liquid is allowed to drain off by keeping the outlet stopcock of the column open. Virus particles get adsorbed onto the surface of the material. Water flows out and with it some of the impurities. The adsorbed virus particles can be reclaimed by washing the column with a large volume of a specific medium such as $MgCl_2$ solution of an ionic concentration higher than that of the viral suspension. The virus particles come out because they have greater affinity for the washing medium. The reclaimed virus is purer than it was earlier. The presence of virus could subsequently be detected in the outflowing fluid (eluate) which is collected in small volumes. Ion exchange resin like, Dowex I have also been successfully used. Separation on columns of neutral substances like silica gel, calcium phosphate (Philipson 1967) and magnesium pyrophosphate have also been reported.

Solvent Extraction

Purification of viral preparations may also be achieved by what is known as partition between solvents. It is well known that a substance

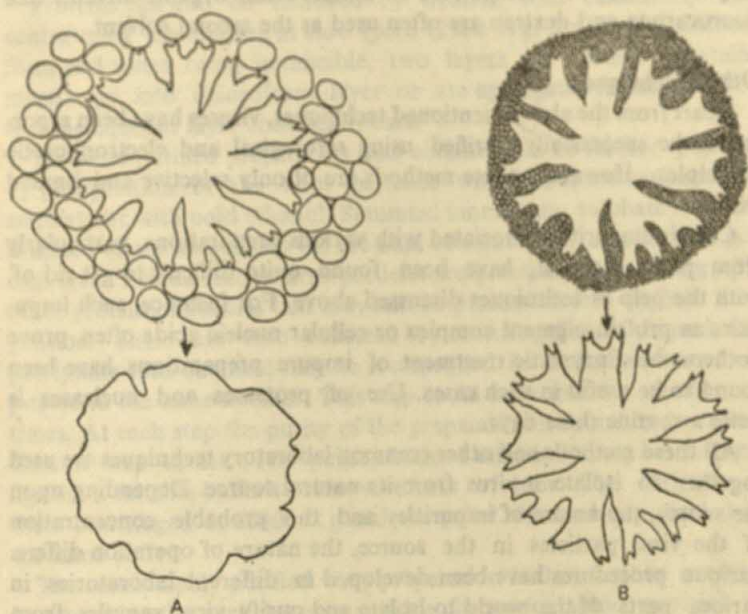


FIG. 2.6. A diagrammatic comparison between the resolution of image of an object by electron microscopy and by light microscopy. The object illustrated here is desmid. Average wavelength of light radiations are comparatively large. Therefore, they cannot penetrate into the sharper details of the desmid. The image formed as a consequence is less resolved and all that can be seen is a vague outline (A). Electron beams, on the other hand, have much shorter wavelength and can easily lighten the sharper features of the object. The result is a much clarified image (B).

could be soluble in more than one solvent. Usually the solubility of the material in these solvents (the amount that can be dissolved in a fixed volume of the solvent) also differs. In other words, a substance is more soluble in one solvent than in the other. If we wish to purify a virus preparation containing some impurities, then the suspension is first mixed with one of the solvents. Since the suspension is in water, it forms one of the solvents. The second solvent has to be immiscible with water. The entire mixture is then shaken vigorously and allowed to stand. If the impurities are more soluble in the second solvent than they are in water, they gradually pass into it. Virus particles remain in the water medium. In this manner, impurities are eventually removed and the viral preparation becomes purer and purer. Since the two solvents are immiscible, the separation of pure

suspension and the impurities is almost total. Organic solvents like fluorocarbon and dextran are often used as the second solvent.

Other Techniques

Apart from the above mentioned techniques, viruses have been reported to be successfully purified using serological and electrophoretic principles. However, these methods are of only selective and limited utility.

Certain impurities associated with various preparations, particularly from plant material, have been found quite difficult to get rid of, with the help of techniques discussed above. For instance, such impurities as protein pigment complex or cellular nucleic acids often prove bothersome. Enzymatic treatment of impure preparations have been found to be useful in such cases. Use of proteases and nucleases is quite a routine these days.

All these methods and other common laboratory techniques are used together to isolate a virus from its natural source. Depending upon the source, the nature of impurities and the probable concentration of the virus particles in the source, the nature of operation differs. Various procedures have been developed in different laboratories in various parts of the world to isolate and purify virus samples from plant and animal sources. We shall consider here briefly the basic features of these procedures as applied to different sources.

ISOLATION AND PURIFICATION OF PLANT VIRUSES (Modified after Markham and Smith 1949)

The infected plant materials or tissue and cell cultures harbouring virus particles are first collected. The infected tissues are then cut into small pieces and then homogenized by any available means. A suitable buffer is used as the extracting medium. The extract obtained is then filtered through a piece of fine cloth to separate the cell debris. The filtrate is then centrifuged at low speed ($2500 \times g$) to remove the remaining cell residues.

The crude preparation obtained in this manner contains soluble impurities like plant proteins. Gummy substances may also be present. Plant proteins could be selectively precipitated at this stage by heating crude preparation upto 55°C . Most viruses are found to remain stable even at this temperature. However, some may get destroyed. This treatment, therefore, should be applied with caution. Selective precipitation of the bulk plant protein could also be achieved by treating the suspension with a specified amount of ethanol.

Proteins can also be removed by treating with chloroform and centrifuging the mixture at slow speed ($2500 \times g$) for 20 min. Chloroform and water being immiscible, two layers are formed. Proteins mostly go into chloroform layer or are aggregated at the interface and the aqueous layer contains viruses.

The semi-purified preparation thus obtained can be further purified by precipitation of the virus particles with 30 per cent ammonium sulphate or with cold ethanol. Saturated ammonium sulphate solution is added to the suspension till the final concentration reaches 30 per cent level. Proteinaceous virus particles are precipitated out along with other protein impurities that may still be present in the original suspension. These are then collected by centrifugation. If desired, the precipitate thus obtained may be resuspended in buffer and reprecipitated in the same manner. This step could be repeated a number of times. At each step the purity of the preparation is found to be more than it was earlier. The pellet obtained ultimately may be dialysed against a suitable buffer to remove excess of ions. The dialysate is then centrifuged at $60000 \times g$ and the pellet obtained resuspended in the same buffer.

The considerably purified virus preparation is then further purified by adsorbing the particles on a suitable chromatographic material in a column like that of calcium phosphate or of Dowex I, and then reclaiming them by means of elutant or washing solution. Most of the impurities present till now get removed by this technique. Impurities that cannot be adsorbed by the column simply move out with the outgoing buffer solution. Protein impurities, of course, get absorbed on the column. However, very little portion of it is likely to come out with the washing solution. This is because of the low affinity of these proteins towards the washing solution.

The virus particles present in the washing solution may then be passed through a membrane filter capable of retaining viruses. Such retained particles can easily be resuspended in a small volume of the buffer. This suspension may again be reprecipitated with ammonium sulphate. The precipitate collected by centrifugation has considerably purified virus particles. Repeated and careful precipitation in cold temperature conditions are likely to yield crystalline preparation. In fact, to ensure that the physical characteristics of the viral preparation remain little disturbed, the entire operation should preferably be carried out at low temperature conditions. These may further be purified by density gradient ultra-centrifugation.

It should be remembered that the same process is not equally effective in the isolation of plant viruses from different sources. In

recent years several modified procedures have been developed to meet challenges offered by different situations.

PREPARATION OF ANIMAL VIRUSES

(Modified after Crammer 1963)

Isolation and purification of animal viruses is more complicated. Infected parts of an animal or tissues cultivating animal viruses are first collected. These are then homogenized in a cold with a suitable buffer. On proper homogenization, the tissues get completely disrupted and an uniform suspension is obtained. This suspension is then centrifuged, first at slow speed ($1400 \times g$) and then at high speed ($7000 \times g$) for 20 minutes duration each time. Impurities sediment down and a clear suspension is obtained.

The suspension is then passed through a bacterial filter to get rid of most of the larger suspended particles. The filtrate is then passed through a membrane filter to retain the virus particles. The filtered particles are then resuspended in a small volume of a suitable buffer. The virus preparation at this stage contains some cell proteins and liquids as impurity.

This suspension is then ultra-centrifuged at a very high speed ($20,000 \times g$) for several hours. Virus particles sediment out and a pellet is formed at the bottom of the tube. In certain cases ultra-centrifugation should be done with caution as it might disturb the physical architecture of the viruses. The centrifuged virus particles are then suspended in a small volume of the buffer. At this stage it could be stored for further purification at a later date. For this purpose, the suspension may be evaporated to dryness in vacuum or dried under freezing conditions (lyophilization).

At this stage, the partially purified virus preparation contains a reduced amount of both proteinaceous and non-proteinaceous impurities. The latter, like lipids and carbohydrates, are separated first. Lipids can be removed by treating the preparation with some lipid dissolving but harmless (for virus) organic solvent. Since these solvents are immiscible with the buffer used, the dissolved lipids are all present in the organic solvent. Repetition of this process makes the preparation almost lipid free. Polysaccharides, if present, may be removed at this stage by electrophoresis. It would also be removed by non-exchange chromatography at a later stage.

The sample thus becomes ready for chemical precipitation. This is done by treating it with ethanol or with 30 per cent ammonium sulphate, final concentration, for 12 hours or more. Precipitation can also

be achieved by adjusting the pH of the suspension to the isoelectric point of the viral proteins. This is preferred in certain cases where protein impurities are still there and can only be removed by selective precipitation.

The precipitate is then collected by centrifugation at high speed and resuspended in a small volume of the buffer. It is then dialysed against a suitable buffer. It may be further purified by adsorption chromatography as detailed earlier. Density gradient centrifugation is useful at this stage; virus particles of the same molecular weight may thus be segregated. The preparation at this stage is almost pure chemically. Crystallization may be attempted now. The particles are resuspended as usual, and precipitated under controlled conditions. Precipitates formed gradually crystallize out.

The general procedure outlined above can be used for isolating and purifying viruses from other sources, like insects or bacterial lysates, as well. Individual cases, of course, necessitate modifications.

CRITERIA OF PURITY OF A PREPARATION

When a sample has been purified in this manner the question that comes up is about its purity. This question has two aspects. The purity or the homogeneity status has to be considered from the (i) physico-chemical as well as from the (ii) biological point of view.

Chemically, a preparation may be regarded as the purest when it is available in the crystallized state. Even in suspension, an extremely purified sample shows certain characteristics of its own. For instance, a purified suspension of tobacco mosaic virus shows a very brilliant shine. In most cases an unusual brightness is visible.

Physical criteria like size, shape, solubility and molecular weight are also helpful in establishing the purity status. In recent years, electrophoretic and serological data have been found useful in determining the homogeneity of a sample. However, even in a state of high apparent purity, foreign material like protein molecules of the host may still be present. Absolute purity is, therefore, rarely, if at all, achieved.

Biological purity of virus preparations has also to be ensured. Biological purity relates to the capacity of the preparations to infect a host. If this capacity is lost during the preparation of the sample, it would biologically be impure. This capacity should, therefore, be tested at every step to see if it is still there. With successive stages of purification, the infectivity tends to get diminished with relation to number of observed particles. If proper precaution is maintained throughout the operation, this loss can be substantially checked. How-

ever, absolute constancy in relation between infectivity and number of particles at every range is rarely achieved. (See Knight 1974).

CHARACTERIZATION OF VIRUSES

Purified samples thus obtained are now ready for various morphological and chemical investigations. The different techniques utilized for this purpose include electron microscopy, X-ray crystallography, sedimentation analyses and various physico-chemical procedures.

Electron Microscopy

Conception about viruses as agents never to be seen continued almost upto the forties of the present century. Earlier attempts to see viruses with even the most powerful of optical microscopes of the day were largely unsuccessful. This was so because visible light radiation with an average wavelength of about 5500 Å (Angstrom unit, equivalent to 10^{-8} centimeter) were unable to lighten the finer and detailed aspects of virus particles, which are comparatively smaller in size. The light wavelengths are relatively long. Therefore, particles having smaller size cannot be properly resolved *i.e.* distinctly and separately identified. The light wavelengths pass over the object without getting disturbed in any way. This was more or less in the manner of huge sea tides submerging small fisherman's boats (Fig. 2.7) (Plate III).

Obviously, for proper resolution of viruses, radiation with smaller wavelengths was necessary. Suitable microscopes designed to use these radiations were also needed. Ultra-violet microscopes were developed and used to observe viruses directly. These microscopes utilize ultra-violet rays which have an average wavelength of about 2200 Å. With these, it was possible to observe some of the larger viruses. Barnard in 1933 reported the resolution of some animal viruses with the help of such microscopes.

The real breakthrough came with the development of electron microscope by Knoll and Ruska in 1931. These instruments do not use electro-magnetic radiation with longer wavelength. Instead, strong electron beams are projected from a suitable source to resolve the object under observation. The wavelengths of such electron beams are very small, often less than 1 Å. On the other hand, the distance between different atoms in a molecule is more than that. Therefore, it is theoretically possible to obtain resolutions at the atomic level with the help of these. Once resolution at such a fine degree is obtained, it is possible to have enlarged and magnified images to the desired extent. Detailed features of the morphology of viruses could at least be

visualized.

There are certain special features in the operation of electron

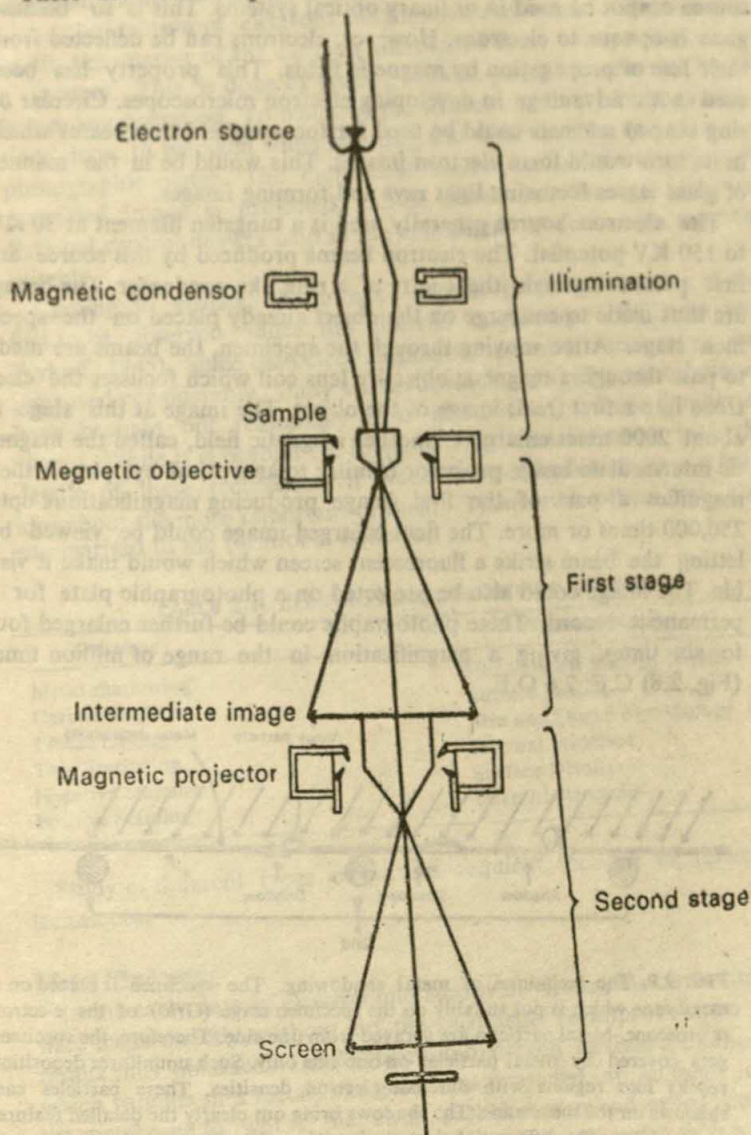


FIG. 2.7. Path of electron beam through an electron microscopic system. Ring like magnetic fields act as various types of lenses and direct the path of movement of electrons. Image formed can be seen on a fluorescent screen placed across the electron path.

microscopes. Short wavelengths radiations produced by high energy source cannot be used in ordinary optical systems. This is so because glass is opaque to electrons. However, electrons can be deflected from their line of propagation by magnetic fields. This property has been used with advantage in developing electron microscopes. Circular or ring shaped magnets could be used for focussing electron beams which in its turn would form electron images. This would be in the manner of glass lenses focussing light rays and forming images.

The electron source generally used is a tungsten filament at 30 KV to 150 KV potential. The electron beams produced by this source are first passed through the centre of a ring like condenser. The beams are thus made to converge on the object already placed on the specimen stage. After moving through the specimen, the beams are made to pass through a magnetic objective lens coil which focusses the electrons into a first (real) image of the object. The image at this stage is about 2000 times enlarged. Another magnetic field, called the magnetic intermediate image projector (similar to an optical eye piece) then magnifies a part of the first image producing magnifications upto 250,000 times or more. The final enlarged image could be viewed by letting the beam strike a fluorescent screen which would make it visible. The image could also be projected on a photographic plate for a permanent record. These photographs could be further enlarged four to six times, giving a magnification in the range of million times (Fig. 2.8) C.F. 2.6 O.E.

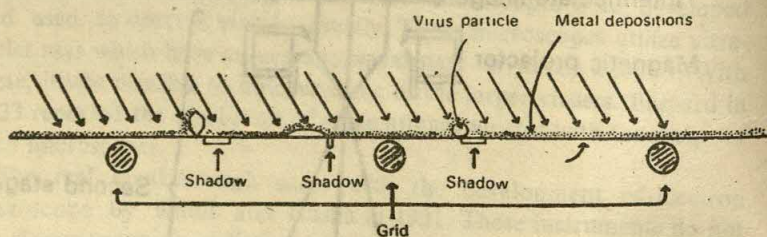


FIG. 2.8. The technique of metal shadowing. The specimen is placed on a membrane which is put suitably on the specimen stage (Grid) of the electron microscope. Metal particles are sprayed from one side. Therefore, the specimen gets covered by metal particles on one side only. Such ununiform deposition results into regions with different electron densities. These particles cast shadows on the membrane. The shadows bring out clearly the detailed features of the object. The differential electron densities also increase the contrast of the image.

Unlike images produced by visible light, electron images are produced mainly by the scattering of electrons. Scattering is produced when

electrons strike atoms. Different atoms forming a molecule produce scattering to different degrees. Therefore, there is more contrast in the image and clear pictures are produced.

As the motion of electrons cannot smoothly occur in the presence of air, the interior of the electron microscope must be maintained at a vacuum. This is done by placing suitable pumps to remove the air before any operation. To maintain the vacuum within, proper precautions have to be taken while inserting and removing the object or the photographic plates. The fluorescent screen on which the second magnified image is projected, can be viewed only through potholes. External control devices help in the adjustment of focussing magnets suitably.

Coming to viruses, the tobacco mosaic particles were one of the first to be observed under the electron microscope (Williams and Wykoff 1945). Since then electron microscopy related technology has improved considerably. Many studies involving a variety of techniques have brought out reasonably clear pictures of a large number of viruses. While it is beyond the scope of this discussion to go into the details of these techniques, it would be worthwhile to have a brief idea about the more important of them. These and others are also summarised in the form of a Table 2.1

TABLE 2.1. ELECTRON MICROSCOPY OF VIRUSES

| <i>Technique</i> | <i>Nature of Study</i> |
|-------------------|-----------------------------|
| Metal shadowing | Morphology; Surface Details |
| Carbon Replica | Surface Details |
| Freeze Drying | Size and Shape: Morphology |
| Thin Sectioning | Internal Structure |
| Negative Staining | Surface Details |
| Positive Staining | Internal Structure |

Study of different types of viruses requires the use of different techniques.

Metal Shadowing

In the early days of electron microscopy of viruses, the observations were made directly, without any pre-treatment. To improve upon the results thus obtained, Drs Williams and Wykoff devised a method in which vapours of a metal, like gold were projected at an angle onto the membrane to which the virus particles were adsorbed during observation. The vapourized metal coated the particles with a thin layer. However, such coating would be ununiform, with the exposed

side getting more of the metal depositions.

Because of this coating, the particles become opaque to the electron beams and their outlines become more clear. The net result is that a shadow is formed on the side opposite to the direction of the metal vapours. These shadows provide images that are much better than those obtained with unshadowed particles. Morphology and surface details can be deduced very clearly from a study of the ununiformly shadowed particles. (Fig. 2.9) (Williams and Wykoff 1943).

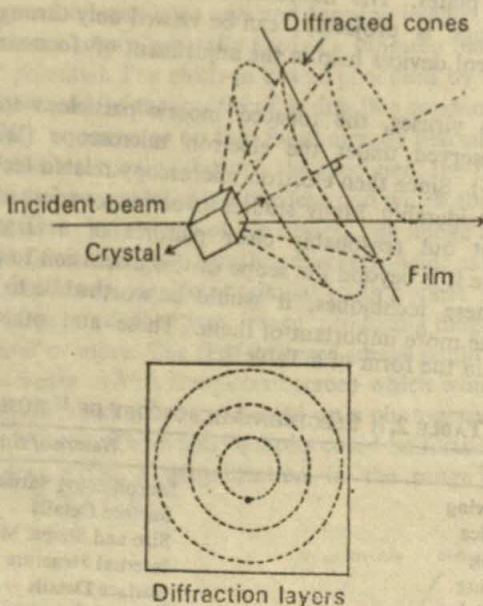


FIG. 2.9. X-ray crystallography of a virus crystal. X-rays are projected on the crystal from a suitable source. The rays traverse through the crystal; some of them pass through straightway. Others face the molecular arrangement, and instead of going straight, bend sideways and come out at different angles. This is known as diffraction. Since the crystals are symmetrical, the diffracted rays also come out in a symmetrical manner. Under certain experimental conditions the diffracted rays come out in cones (top). When projected on a suitably placed photographic plate, a clear pattern emerge (bottom). Concentric circular rings can be seen. X-ray crystallographers can properly interpret such patterns and can work backwards to build up the architecture of the crystal.

Negative and Positive Staining

It was observed that when virus particles are treated with electron opaque chemicals (through which electrons cannot pass) their electron density increase. This is due to the deposition of the chemical on the

virus particles. However, such deposition is not uniform. This leads into an increase in the contrast of the particles. Osmium tetroxide and sodium phosphotungstate are two such chemicals. Using the latter, Drs Brenner and Horne (1959) devised a staining technique called *negative staining*.

In this method, the virus particles are mixed with the salt solution and spread on a collodion membrane. The solution surrounding the particles permeates between the individual particles and the membrane. It also enters into the uneven surface of particles. Some parts of the particles remain unaffected. When such uniformly penetrated particles are seen through the electron microscopes, the picture appears more clear and distinct, than it is possible to obtain otherwise. In fact, it is the non-deposition of the salt particles at certain areas which makes the particles clearly visible. Hence the name of the procedure. Surface details of the particles are very well studied by this method.

Certain other chemicals like uranyl salts and ferritin get selectively adsorbed by the various components of a virus particle. Uranyl acetate is preferentially adsorbed by the nucleic acid part. On the other hand, ferritin is specifically adsorbed by viral proteins. Deposition of these molecules on the components of a virus particles increase their resolution. This is basically achieved by enhancing the contrast of the particles. We can, therefore, say that these chemicals are directly 'staining' the virus particles. For this reason this method of increasing the resolution of the virus particles is called *positive staining*. This is most suitable for studying internal details.

Other Techniques

Of the other techniques used in microscopic studies of viruses *thin sectioning* is helpful in studying the particle within the host cell. Also, it is useful for studying the crystal structure. *Freeze drying* techniques are used to prevent any possible distortion to the particles while studying them. This method, thus helps in getting a correct idea about the shape of the particles. Preparation of *carbon replica* similar to plaster of Paris moulds, are prepared in many cases to bring out the surface characteristics of virus particles (See Smith 1962).

X-ray Crystallography

Crystals constitute a well recognized feature of the inanimate world. In general, crystals consist of a regular arrangement of atoms repeated over and over again to fill their volumes. Such arrangements are aligned in all the three dimensions. This three dimensional arrangement is ultimately responsible for giving a crystal its charac-

teristic features.

The individual arrangements of atoms in a crystal are called *lattices*. A correct idea about the internal architecture of a crystal can be had only when the alignment of its lattices are completely elucidated. The technique of X-ray crystallography has been extensively and successfully used in the past few decades for this purpose.

Once it was clear that viruses could be obtained in a crystalline state, it was realised by virologists that the fundamental characteristic of a virus crystal must be similar to that of other natural crystals. Now, if that were so, the possibility of elucidating the structure of virus crystal for utilising this technique was obvious. In course of their investigation, it was shown by Prof. J.D. Bernal during the forties and later by Drs Sidney Brenner and Rosalind Franklin, among others, that it could indeed be done.

X-rays are a kind of electromagnetic radiation. These rays have very small wavelength. In fact, these are often shorter than the distance between the different atomic lattices forming a crystal. For this reason X-rays are able to penetrate through the space in between them.

X-rays from a suitable source are allowed to pass through a crystal placed suitably on its path. A few rays just traverse through without any change in their trajectory (plane of motion). However, a major part of the rays encounter the atomic lattices and are unable to pass straight through. What then happens is that the oncoming rays strike the rows of atoms, are sort of reflected, and then bend to bypass the barrier in front. Such bending is called *diffraction*.

While passing through a crystal X-rays are diffracted at specific angles and emerge out forming patterns. These angles of emergence of diffracted rays and the resultant pattern can be correlated with the arrangement of lattices in a crystal. X-ray diffraction patterns, as they are called are thus helpful in elucidating the crystal architecture.

Figure 2.10 diagrammatically depicts a hypothetical X-ray crystallographic analysis of a virus crystal. The Fig. 2.10 shows how incident beam is allowed to pass through one face of the crystal. You will note that some of the rays pass straight through. Some other rays are shown to be getting diffracted at various angles and emerging out as cones. Since the angles of diffraction are different, the cones formed are of different sizes. When suitably projected on a X-ray film, these emerging cones appear as circular rings, arranged one within the other (the bottom diagram). X-ray crystallographers can work backwards and construct the structure of a crystal from their rings

This technique has been used extensively in recent decades to elaborate the architecture of virus crystals. Also our current concepts re-

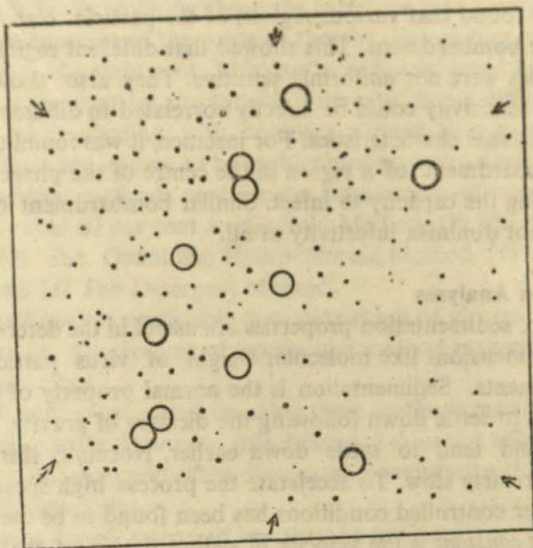


FIG. 2.10. Enumeration of viruses by latex method. The viral suspension to be counted is mixed with polystyrene latex particles of known concentration. The mixture is then sprayed in droplets on a supporting membrane, dried and shadowed. The figure shows one such droplet. This contains many small particles and a few larger ones. The smaller ones are viruses and the larger ones the latex particles.

There are 220 viral and 11 latex particles in the droplet. The latex concentration per ml in the original sample was 3.2×10^{10} . Therefore, the number of virus particles in the sample is $220/11 \times 3.2 \times 10^{10} = 6.4 \times 10^{11}$ particles per ml. Average of several droplets gives a reliable estimate.

garding the nature of the alignment of different viral components are entirely due to such studies. Its continued use is bound to bring out many more unknown features of the virus morphology.

Deuteron Bombardment

Another interesting but not so commonly used technique involves exploring virus particles with high energy radiations. This technique was developed during the forties by Prof. Earnest Pollard and his co-workers at the University of Yale in the USA. They have claimed much success in elucidating the ultrastructure of a virus particle with the help of this technique.

Using highly energetic deuteron particles emanating from a cyclotron (a machine designed to break up bigger atoms into smaller ones; as a consequence of the process high energy particles like deuterons are given out as by-product). Prof. Pollard bombarded bacteriophage

particles and found that various regions of the particle reacted differently to the bombardment. This showed that different regions of the phage particles were not uniformly sensitive. They also showed that difference in sensitivity could be directly correlated to difference in the regional functional characteristics. For instance, it was found that continuous bombardment of a region in the centre of the phage particle led to its losing the capacity to infect. Similar bombardment of other regions did not diminish infectivity at all.

Sedimentation Analyses

Analyses of sedimentation properties are useful in the determination of physical dimensions like molecular weight of virus particles and their components. Sedimentation is the normal property of particles in suspension to settle down following the dictates of gravity. Heavier particles would tend to settle down earlier. Normally this process would be extremely slow. To accelerate the process high speed centrifugation under controlled conditions has been found to be useful. The *sedimentation constant* is the velocity of sedimentation of the suspended particles in a centrifugal field. This value is generally denoted as (S) or *Svedberg Unit* after its discoverer. Both the relative and the absolute values of sedimentation constant can be obtained utilizing preparative and analytical types of ultra-centrifuges respectively. The molecular weights of the particles under investigation can then be calculated from the value of *S*.

Chemical Determination

Detailed chemical analyses of viruses can be done using a large array of physico-chemical techniques. Utilizing them it has been possible to segregate the major chemical constituents of different classes of viruses. Not only that, chemistry of the individual viral components, like nucleic acid and coat proteins have also been substantially elucidated. However, it should be remembered that these techniques are all generally applicable to these cellular bio-chemicals, irrespective of their source. We shall here briefly take note of the techniques as applied to the analyses of viral components (See Knight 1974, Sanger *et al.* 1977 for details).

Proteins

Viruses which are, chemically speaking, nucleo-protein particles, must be suitably treated to separate out the proteins and the nucleic acid moieties. Often it has been observed that the preparation and purification of one part is satisfactorily achieved only at the expense

of the other. While isolating them, the basic idea is to disrupt the quaternary configuration of the proteins thereby dissociating them from the nucleic acid component. This can be easily achieved by treatment with acid or alkali or by any suitable detergent. Some difficulty is encountered while dealing with complex viruses like bacteriophages whose proteins are known to be multicomponent structures.

The important methods used for the preparation of viral proteins are: (1) The cold 67 per cent Acetic Acid Method, (2) The Mild Alkali Method, (3) The Guanidine Hydrochloride Method, (4) The Phenol Method, and (5) The Detergent Method.

These methods are applicable to a wide range of viruses. However, the degree of effectiveness of a particular method may vary with the nature of the virus.

The viral protein prepared by using these methods may be different from its native state. However, this difference does not affect its chemical nature but may and often does cause denaturative changes in its physical characteristics.

The protein sample prepared can then be analyzed for (1) Amino acids, (2) Protein end group, *i.e.* the C-terminal end and the N-terminal end, (3) Protein subunits and, (4) Amino acid sequence. The data obtained with respect to the above item go a long way towards chemical characterization of viruses.

Nucleic Acids

The nucleic acids, whether RNA or DNA form the other major constituent of viruses. The preparation of viral nucleic acid, which remains enveloped within the protein coat, necessitates careful handling. One persistent problem in the isolation of viral nucleic acids is the presence of contaminating proteins. Host cell nucleases have also to be counteracted to prevent any loss to the viral nucleic acids. Several isolation and purification procedures have been developed from time to time to obtain purified viral nucleic acid preparations. Some of these are:

- (1) Hot salt method NaCl
- (2) Detergent method
- (3) Phenol method
- (4) Alkaline method.

It was earlier thought that the viral nucleic acids lost some of their stability during preparation, thereby becoming less infective. However, it is becoming increasingly clear that the loss is not appreciable.

Viral nucleic acid preparations can further be analyzed to bring out their chemical characteristics. These analyses may be done at various

levels. Thus, the nucleic acid preparations may be analyzed for

- (1) Base ratios by hydrolysis followed by paper chromatography.
- (2) Base ratios from buoyant density values.
- (3) Base ratios from thermal denaturation values.
- (4) Nucleotide ratios by hydrolysis followed by Column Chromatography.
- (5) Polynucleotide end group.
- (6) Nucleotide sequence analyses.

The secondary and higher structure of the viral nucleic acids can be analyzed by using such physical methods as spectrophotometry and analyses of sedimentation, optical rotation, viscosity and X-ray diffraction patterns.

Apart from proteins and nucleic acids the other chemical constituents reported in viruses are carbohydrates and lipids. The general techniques developed for preparing and analyzing carbohydrates and lipids from biological sources have been successfully employed in the case of viruses as well.

ENUMERATION OF VIRUSES

Enumeration of virus particles in a suspension is considered very important. This is because the knowledge about the number of particles present in a suspension becomes a prerequisite for further studies. Information regarding the relationship between infectivity and number of particles is also significant.

Counting by Direct Electron Microscopy

Often counting is simply done by direct observation under electron microscope. One widely used procedure is called *Latex droplet method* (Backus and Williams 1950). In this method a known volume of the suspension is spread on a cellophane film after being mixed with known number of polystyrene latex droplets. The film is dried and observed under the microscope. Suitable techniques like metal shadowing, increases the resolution of the particles.

Virus particles only get coated with the latex and are seen very clearly. Free latex particles, which are comparatively larger in size are also visible side by side. Both the types of particles are counted and their relative proportions known. Since the concentration of the latex particles sprayed as well as the original concentration of the latex particles per ml. is predetermined, it is easy to calculate the total concentration of virus particles (Fig 2.10. c.h. 2,9 of o.e.).

Plaque Assay

Another method of enumerating viruses is the *Plaque Assay*. In this method a suspension culture of appropriate host cells is prepared initially and later cultured in petri plates containing suitable agar based media. Such cultures are then inoculated with aliquots of the virus titre to be enumerated. On proper incubation virus particles start infecting the host cells. One susceptible host cell is infected by a single virion. After some time, disintegration of the host cells occur resulting in liberation of more virus particles. These, in their turn, infect host cells in the immediate vicinity and destroy them as well. In the meanwhile, the cells that are not infected continue to grow and divide normally. Thus, soon transparent, visible, circular areas appear on the beneficial lawn in the plate. These are called plaques (Plate I)

Since each virion of the original suspension causes one plaque, the number of virus particles in it can be determined with ease. For example, let us suppose 1 ml. of the original suspension was diluted 10^4 times before being distributed with the host cell culture; and let us say, 42 plaques were formed. Obviously, there were 42×10^4 particles per ml. in the stock. In this method, virus suspensions are often mixed with the host cells prior to incubation. Plaque assay has been most effective in the counting of bacterial viruses.

Acid End Point Method

It is well known that vigorously growing tissue or host cell suspension turns acidic the medium in which they are growing. This feature is taken advantage of in this method. It involves measurement of any change in the acidity of the suspension and correlation of the data with standards prepared earlier. Here, the idea is to determine the highest dilution of a suspension which would be capable of causing infection. Thus a medium which is not acidic to the normal level would indicate the presence of dead cells. When virus particles are cultivated with the tissue suspensions, death could be due to viral infection alone.

Supposing we wish to find out the number of infectious virus particles present in a particular suspension. Initially, it is diluted, let us say, 10^4 and 10^5 times, separately. Both the dilutions are then made to infect identical host cell suspensions. Let us assume that with the former no discernible acidity developed whereas with the latter the medium became acidic to the normal level. This shows that when the original suspension of virus particles was diluted 10^5 times there was not even a single virion in it. The more concentrated 10^4 dilution, on the other hand, contained at least one virion. Therefore,

the original suspension had between 10^4 and 10^5 virions. There are several procedural modification of this procedure which are in use in various laboratories. Two common procedures are the metabolic inhibition test and the 50 per cent end point test. These methods are not, however, very precise and need many replications to arrive at a satisfactory result.

Haemagglutinin Assay

For containing viruses is the *Haemagglutinin assay*. Many viruses are capable of agglutinating red blood cells. This phenomenon is known as agglutination or sticking together of red blood cells, and the agent responsible, Haemagglutinin. A virus particle attaches simultaneously to two red blood cells and bridges them. Such formation of aggregates can be detected in a number of ways and afford a simple method for virus enumeration.

One of the simplest and most commonly used haemagglutinin assay procedure is the *pattern method*. This is based on the observation that when the suspension of blood cells and the virus suspension is left undisturbed in a small tube for several hours, non-aggregated and aggregated cells behave differently. While the former sediment to the bottom of the tube and then roll toward the center, the latter only sediment to the round bottom but do not roll. The aggregated particles rather form a thin film that is very characteristic of a particular virus.

The actual assay is carried out by an end point procedure. Serial dilutions of the virus sample is taken in replicates and mixed with a standard dilution of red blood cells (usually 10^7 per ml). The last dilution showing complete hemagglutination is regarded as the end point. This value could be regarded as an estimate of the viral population (Fig. 2.11). The titre estimated by this method has, however, an inherent imprecision at least as large as the dilution step used (usually two fold). Colometric measurement of the experimental titres and their calibration on a graph gives more precise information.

Particle Count and Infectivity

Studies on the enumeration of viruses reveal an interesting situation. It has been found that in many cases there is no one to one correspondence between the number of particles as physical entities and particles capable of infecting a cell (Table 2.2). In the case of tobacco mosaic virus the frequency of occurrence of an infective particles may be 10^6 . While some of the methods described above tell us about the number of particles others indicate the number infective units under the given conditions. The data obtained should, therefore, be assessed

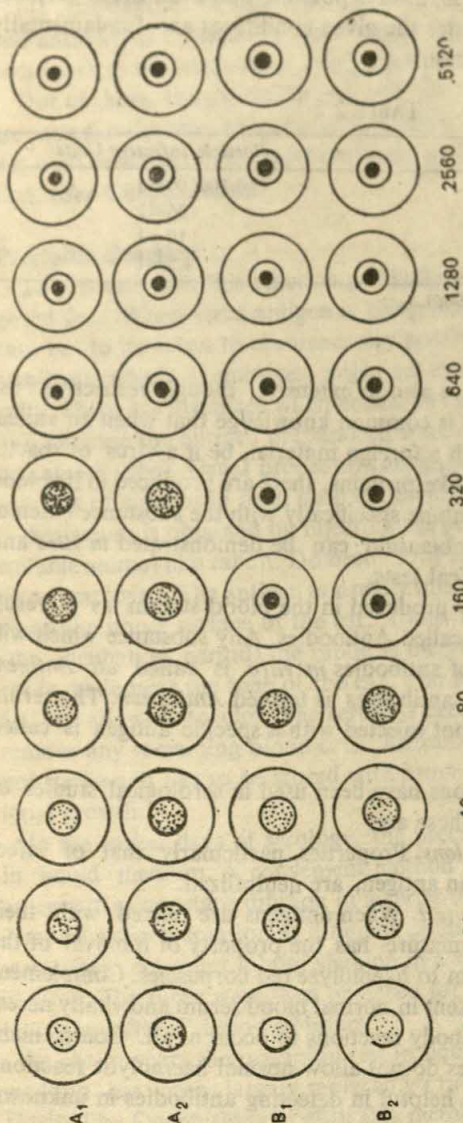


FIG. 2.11. Counting of viruses by haemagglutination assay. The method depicted here is the pattern method which is one of the several in use. Two samples A and B were diluted serially by using two-fold steps (10, 20, 40, 80 and so on.) 0.5 ml of each dilution was mixed with an equal volume of red blood cells suspension (10^7 /ml). Each mixture was placed in a cup drilled in a clean plastic plate and left for 30 minutes at room temperature. Each assay was done in duplicate. Sample A caused complete haemagglutination until dilution 320. Sample B did the same until dilution 80. This meant that these dilutions had just enough viruses to agglutinate the blood cells present. Now the number of red blood cells per ml of the reaction mixture was 10^7 . Since one virus particle is capable of agglutinating two blood cells simultaneously, the number of viruses required for agglutinating 10^7 cells would be 5×10^6 . Therefore, sample A had $320 \times 5 \times 10^6$ virus particles and sample B, $80 \times 5 \times 10^6$ particles. However, the populations are expressed simply in haemagglutinin units. Accordingly, samples A and B had 320 and 80 units respectively.

with caution. It is, of course, always possible that even those particles which are non-infective under the given conditions are fundamentally capable of successful infection.

TABLE 2.2

| <i>Virus</i> | <i>Particle Infective Units</i> |
|------------------------------|---------------------------------|
| TMV | 100,000=1 |
| Polio | 36=1 |
| Influenza | 10=1 |
| Bacteriophage T ₂ | 1.5=1 |

Adapted after Waterson (1966).

Serolog

Serological techniques are also in extensive, though restrictive, use in the study of viruses. It is common knowledge that when an animal (vertebrate) is infected with a foreign material, be it a virus or bacterium or a macromolecule like proteins, there are produced in its blood stream proteins which combine specifically with the substance entered. This act of specific combination can be demonstrated *in vitro* and forms the basis of serological tests.

The proteins specifically produced in the blood stream as a result of external stimulus are called Antibodies. Any substance which will stimulate the production of antibodies *in vitro* is called an *Antigen*. Blood serum containing antibodies is termed *Antiserum*. The serum obtained from an animal not injected with a specific antigen is called Normal serum.

Antigen-antibody reactions have been used in serological studies of viruses in several ways. These are:

(1) *Neutralization reactions*. Properties, particularly that of infection, of a virus acting as an antigen, are neutralized.

(2) *Complement fixation test*. When antigens are mixed with their specific antibodies, the mixture has the property of removal of the power of the normal serum to haemolyze red corpuscles. Complement is a neat labile protein present in normal blood serum and vitally necessary for some antigen-antibody reactions to occur at all. Complement when 'fixed' by antibodies do not allow normal haemolytic reactions to occur. This can thus be helpful in detecting antibodies in unknown serum.

(3) *Precipitin reaction*. When a virus comes in contact with its specific antiserum taken in saline at different dilutions, and warmed in a water-bath, a precipitate is formed. The antibody is referred to as the precipitin and the reaction as precipitin test.

(4) *Anapylaxis*. In this type of test, the union between antigen and antibody is detected by reaction offered by animal tissues. It is an *in vivo* system of detection of antigen-antibody reaction.

Out of these, the precipitin reaction and complement fixation test are extensively used in virological studies. Additionally, this property has been exhibited in the new universal process of artificial immunization. (See Chapter Eight).

Precipitin Reaction

To proceed with this reaction purified antigen (virus) has to be prepared first. When virus antigen is being prepared from tissues extreme care has to be taken to eliminate any possible protein or similar contaminant which themselves might act as antigen and confuse the results. There are instances when erroneous conclusions have been drawn only because the antigen preparation were contaminated (see Van Regenmortel 1966). Usual procedures adopted for preparation of purified samples of virus have been outlined earlier.

Antiserum is obtained by injecting the purified antigen (virus) into a suitable animal like rabbit. Domestic fowl and also the horse are sometimes employed. The antigen is usually injected into the vein which runs along the upper surface of the ear. About two weeks after injection (the incubation period) the rabbit is bled from the other ear. A small cut is made near the base of the ear. The blood is collected in a tube and is allowed to clot. The serum is decanted off and centrifuged to remove any remaining blood cells. Antiserum prepared in this way is not sterile and has to be stored with proper precaution to prevent bacterial growth.

In the earlier days of serology, the precipitin test was carried out in liquid medium. A convenient method was to use 1 ml. samples of antiserum at constant dilution in a series of test tubes to each of which 1 ml. of antigen solution at different dilutions was added. The tubes were then set after a brief shaking in a water bath with temperature at 50°C. The tubes were then observed for the appearance of precipitate often after a period of hours.

In recent years the precipitin test in liquid (usually normal saline) medium has been largely replaced by precipitin reactions in agar gel. Devised by Onchsterlony (1950), this method is known as the Plate test. In this method agar gel is prepared in a petri plate with a number of wells in it. Usually one well is bored in the centre and six or seven in the peripheral regions. The known antigen is placed in the central well. The unknown and a suitable control are placed in the peripheral ones. The preparation is kept moist and within a few hours the pro-

tein solutions diffuse through gel. A clear line of precipitate is produced where antigen and antibody meet. The precipitate can be made clearly visible by staining with a dye.

Of the other tests, the complement fixation test (CFT) and anaphylaxis find extensive application in animal virology, particularly medical virology. The role of CFT in elucidation of the problem of possible viral oncogenesis cannot be overemphasized.

Serological data have also been utilized for finding out relatedness or otherwise amongst viruses. As early as in 1937 Bawden and Pirie reported that the viruses then known as Cucumber viruses 3 and 4 were in fact related to tobacco mosaic virus. On the other hand, they reported that tobacco necrosis virus was in fact comprized of a number of serologically distinct virus types. Since then serological relationships between otherwise considered distinct virus types have been reported from time to time. For instance, the serological relationship between pea mosaic and bean yellow mosaic viruses.

Chapter Three

CHEMISTRY OF VIRUSES

A probe into the chemistry of viruses could start almost four decades after their discovery. One reason for such inexplicable delay in investigating these basically chemical entities was the subconscious *a priori* acceptance by scientists of the day of the microbial nature of viruses. The chemical connection was, more often than not, consciously overlooked.

Once their particulate nature was partially established by Max Schlessinger in 1933, the *chemistry of viruses* was sought to be elaborated in further detail. Schlesinger isolated a large bacteriophage, WLL, infecting the bacterium *Escherichia coli*, by differential ultra-centrifugation. On chemical investigation, he found proteins and phosphoric acid to be associated with these particles. He tentatively suggested the presence of nucleic acid in them. His conclusion that these bacteriophage particles were possibly nucleoproteins was remarkable though often overlooked.

The precise chemical identity of a virus could be determined only after tobacco mosaic virus (TMV) was crystallized by Stanley in 1935. Later, Bawden, Pirie and their associates, were the first to establish the nucleoprotein nature of three strains of TMV. They also showed that the nucleic acid component was ribonucleic acid (RNA) (Bawden and Pirie 1937).

Thus, in the words of C.A. Knight (1974), 'the chemical era of virology was launched.' During the last four decades, an enormous amount of data have been collected on various aspects of the chemistry of different classes of viruses. In the following pages we shall attempt to present a summarised glimpse of the world of chemical virology.

CHEMICAL COMPOSITION OF VIRUSES

Chemically, viruses are nucleoproteins. Data reveal that a majority of viruses consist of two components. One is a single usually linear molecule of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The other is an outer proteinaceous covering or sheath sur-

rounding the inner nucleic acid molecule. However, some of the larger animal and bacterial viruses are reported to contain lipids (Franklin 1962) and carbohydrates (Compans and Choppin 1973). Recent reports suggest that lipids are also associated with even a few plant viruses (Best 1968). The general chemical composition of some plant, animal and bacterial viruses are listed in Tables 3.1, 3.2 and 3.3.

A casual perusal of the chemical composition data of different categories of viruses shows that:

(1) the composition varies, both qualitatively and quantitatively, from virus to virus. There does not seem to be much similarity with respect even to a specific category of viruses, except perhaps the 'T' even series of bacteriophages.

(2) bacterial and animal viruses are mostly DNA viruses whereas plant viruses are in most cases RNA viruses. However there are exceptions in both the cases. For example, coliphage f_2 and influenza virus are RNA viruses and cauliflower mosaic and golden yellow mosaic viruses are DNA containing viruses.

TABLE 3.1. CHEMICAL COMPOSITION OF SOME PLANT VIRUSES
(As percentages)

| <i>Virus</i> | <i>RNA</i> | <i>DNA</i> | <i>Proteins</i> | <i>Lipids</i> | <i>Carbohydrates</i> |
|--------------------------------|------------|------------|-----------------|---------------|----------------------|
| Alfalfa mosaic | 19 | — | 81 | — | — |
| Broad bean mottle | 22 | — | 78 | — | — |
| Carnation latent | 6 | — | 94 | — | — |
| Carnation etch ring | — | — | — | — | — |
| Cauliflower mosaic | — | 16 | 84 | — | — |
| Cucumber mosaic | 18 | — | 82 | — | — |
| Cyanophage-LPP I | — | 40 | 60 | — | — |
| Dahlia mosaic | — | — | — | — | — |
| Golden yellow mosaic | — | 23 | 77 | — | — |
| Pea enation mosaic | 29 | — | 71 | — | — |
| Potato spindle tuber (Virioid) | 100 | — | — | — | — |
| Potato X | 6 | — | 94 | — | — |
| Potato leaf roll | — | — | — | — | — |
| Tobacco necrosis | 19 | — | 81 | — | — |
| Tobacco mosaic | 5 | — | 95 | — | — |
| Tomato bushy stunt | 17 | — | 83 | — | — |
| Tomato spotted wilt | 5 | — | 71 | 19 | 5 |
| Turnip yellow mosaic | 34 | — | 67 | — | — |
| Wound tumour | 23 | — | 77 | — | — |

[Adapted after Shepherd (1968), Fujisawa *et al.* (1968, 1972), Best (1968), Knight (1974), Sarkar (1976), Goodman (1977).

TABLE 3.2. CHEMICAL COMPOSITION OF SOME ANIMAL VIRUSES
(As percentages)

| <i>Virus</i> | <i>RNA</i> | <i>DNA</i> | <i>Proteins</i> | <i>Lipids</i> | <i>Carbohydrates</i> |
|---------------------------|------------|------------|-----------------|---------------|----------------------|
| Adenovirus | — | 13 | 87 | — | — |
| Equine encephalo myelitis | 4 | — | 42 | 54 | — |
| Fowl plaque | 2 | — | 69 | 26 | 2 |
| Herpes simplex | — | 9 | 67 | 22 | 2 |
| Influenza | 1 | — | 74 | 19 | 6 |
| Poliomyelitis | 26 | — | 74 | — | — |
| Polyoma | — | 16 | 84 | — | — |
| Reovirus | 21 | — | 79 | — | — |
| Rous sarcoma | 2 | — | 62 | 35 | 1 |
| Shope papilloma | — | 18 | 82 | — | — |
| Simion virus 5 (SV5) | 1 | — | 73 | 20 | 6 |
| Tipuia iridescent | — | 13 | 82 | 5 | — |
| Vaccinia | — | 5 | 88 | 5 | 2 |

[] Adapted after Knight (1974); Wyatt & Cohen 1953.

TABLE 3.3. PERCENTAGE COMPOSITION OF SOME BACTERIAL VIRUSES

| <i>Virus</i> | <i>RNA</i> | <i>DNA</i> | <i>Proteins</i> | <i>Lipids</i> | <i>Carbohydrates</i> |
|--------------------------------|------------|------------|-----------------|---------------|----------------------|
| Coliphage f_2 | 30 | — | 70 | — | — |
| Coliphage M_{13} | — | 12 | 88 | — | — |
| Coliphage ϕ X 174 | — | 26 | 74 | — | — |
| Coliphage T_4 | — | 55 | 40 | — | 5 |
| Coliphage T_2 | — | 50 | 50 | — | — |
| Coliphage lambda (λ) | — | 56 | 44 | — | — |

After Butler (1970); Knight (1974); Wyatt and Cohen (1953).

The observed variation in the chemical composition of different classes of viruses is, however, not without significance. It is now widely recognised that the different chemical ingredients are individually and specifically related to the various functional aspects of a virus. Indeed, it may not be out of the mark to say that the observed quantitative variations have a significant relationship with the physiology of viruses. These aspects will be discussed in a subsequent chapter (See Chapter Five).

Viriods

There are reports which indicate that a viral nucleic acid may remain entirely naked in a host cell, without being ensheathed by a protein coat. Possibly, such nucleic acid moieties are associated with the host cell chromosome and are endogenously passed on from one generation to the next. Under certain condition they may become dissociated from the host chromosome. In a dissociated state they are capable

of replicating leading to increase in their level in the host cell. Such increase often leads to disruption in the physiology of the host cell, sometimes resulting into neoplastic growth. The 'viroid' of potato spindle tuber virus and citrus exocortis virus as also the C-type RNA of murine leukaemia viruses (MLV) are examples for such cases.

The current understanding regarding these enigmatic particles is based mainly on the study of potato spindle tuber disease. T.O. Diener and his associates were successful for the first time, in isolating the causative agent for this disease. They found it to be a naked RNA molecule of very low molecular weight which was capable of self replication. They called these particles *Viroids*.

Recent researches reveal that the *viroid* of PST disease is a single stranded RNA molecule with a molecular weight of 80,000-90,000. It has to also be recognised that these RNA molecules are covalently closed giving it a cyclic appearance. There are evidences to indicate that the RNA strand is bent like a hair pin with considerable lengths of base paired regions. Sometimes the closed strand takes the shape of a dumb-bell while some other time it looks a simple rod.

There remain many unanswered questions regarding origin, mechanism of replication, pathogenicity and transmission of viroids. There are evidences to suggest that the viroids synthesise small molecular weight DNA molecules possible utilising a system like RNA-directed DNA polymerase. This DNA units turn might be synthesising more viroid RNA molecules. It is further suggested that the newly synthesized DNA could remain integrated with host cell DNA, thereby maintaining itself in a latent condition. However how could again become pathogenic remains unclear.

NUCLEIC ACIDS

The nucleic acid moiety is the active, disease specific and host specific infective part of a virus. It should be noted that both the types of cellular nucleic acids viz, the DNA and the RNA are not present together in any one virus. This trait distinguishes viruses from the known living organisms all of which possess both the types of nucleic acids. Viruses possessing DNA are referred to as deoxy viruses, while those possessing RNA as ribo-viruses.

Types of Nucleic Acids

The nucleic acids of most viruses are linear straight chain structures. Some, however, such as those from DNA tumour viruses and a few bacteriophages like *Pseudomonas* phage PM₂ (Espejo *et al.* 1969) and

Rhodospirillum phage RQGP (Pemberton and Tucker 1977) contain cyclic or circular structure. These circles are known to be supercoiled or twisted like a ball of thread (Plate IV).

The straight chain or cyclic structures could be single stranded or double stranded. Accordingly, there could be eight categories of viruses, depending upon nucleic acid types. These are: (i) viruses with double stranded DNA, (ii) viruses with single stranded DNA, (iii) viruses with double stranded RNA, (iv) viruses with single stranded RNA, (v) viruses with double stranded cyclic DNA, (vi) viruses with single stranded cyclic DNA, (vii) viruses with double stranded cyclic RNA, and (viii) viruses with single stranded cyclic RNA. Table 3.4 depicts the nucleic acid type of some viruses.

Nucleic Acid Content per Particle

The nucleic acid content per particle varies from virus to virus. Generally, larger the size of the virus particle, larger is the content of nucleic acid and vice-versa. For instance *vaccinia virus* having a length of 250 nm has 160×10^6 daltons of DNA per particle. Conversely coliphage $\phi \times 174$ with a diameter of 22 nm has 1.7×10^6 daltons of DNA per particle. Greater amount of nucleic acid probably is necessary for the synthesis of more complex and larger structural components of the larger viruses.

The nucleic acid content of a virus can be roughly calculated from the molecular weight of a virus and its nucleic acid percentage. For instance, the molecular weight of tobacco mosaic virus (TMV) has been calculated to be 40×10^6 daltons. Since it contains about 5 per cent RNA, its content can be calculated to be roughly 2×10^6 daltons. The content per particle can further be confirmed by calculating the molecular weight of RNA isolated from TMV particles. Fairly homogeneous RNA preparations were shown to be of molecular weight of approximately 2×10^6 .

A direct correspondence between the molecular weight calculated on the basis of percentage nucleic acid content and the one calculated from isolated RNA gives an idea about the number of nucleic acid molecules. A one to one correspondence means that there is a single, linear or circular, molecule. For example, the molecular weight of a isolated RNA (TMV) is approximately 2×10^6 , calculated on the basis of percentage RNA and particle molecular weight, it comes to about the same. This indicates that there is one molecule of RNA per particle.

Nucleic acids of several plant, animal and bacterial viruses have been similarly shown to occur in their respective particles in one piece.

TABLE 3.4. CHARACTERISTICS OF SOME VIRAL NUCLEIC ACIDS

| <i>Virus</i> | <i>NA type</i> | <i>Strands</i> | <i>Content/ Particle (Daltons)</i> | <i>Nucleotide pair</i> | <i>Approx. number of Genes</i> |
|-----------------------------|----------------|----------------|------------------------------------|------------------------|--------------------------------|
| Adenovirus | DNA | Double | 23×10^6 | 35,000 | 120 |
| Bean mosaic | RNA | Single | 1×10^6 | 3,000 | 10 |
| Coliphage fd | DNA | Single | 1.7×10^6 | 5,500 | 18 |
| Cauliflower mosaic | DNA | Double | 1.7×10^6 | 14,000 | 46 |
| Coliphage lambda | DNA | Double | 32×10^6 | 48,000 | 160 |
| Coliphage MS2 | DNA | Single | 1×10^6 | 3,000 | 10 |
| Coliphage $\phi \times 174$ | DNA | Single, Cyclic | 1.7×10^6 | 5,500 | 18 |
| Coliphage T ₄ | DNA | Double | 130×10^6 | 200,000 | 650 |
| Foot and Mouth Disease | RNA | Single | 2×10^6 | 6,000 | 20 |
| Herpes simplex | DNA | Double | 60×10^6 | 90,000 | 300 |
| Influenza | RNA | Single | 2×10^6 | 6,000 | 20 |
| Mouse encephalitis Murine | RNA | Single | 2×10^6 | 6,000 | 20 |
| leukaemia (AKR) | RNA | | | | |
| Poliomyelitis | RNA | Single | 2×10^6 | 6,000 | 20 |
| Polyoma | DNA | Double, cyclic | 4×10^6 | 6,000 | 20 |
| Potato leaf roll | DNA | Double | 0.56×10^6 | 1,650 | 6 |
| Potato virus X | RNA | Single | 2×10^6 | 6,000 | 20 |
| Potato spindle tuber virus | RNA | Single, cyclic | 9×10^5 | 250 | 1 |
| Reo-3 | RNA | Double | 15×10^6 | 15,000 | 50 |
| Rous Sarcoma | RNA | Single | 10×10^6 | 30,000 | 100 |
| Simian Virus 40 | DNA | Double, cyclic | 4×10^6 | 6,000 | 20 |
| Tobacco Mosaic virus | RNA | Single | 2×10^6 | 7,400 | 20 |
| Tobacco bushy shurt | RNA | Single | 0.2×10^6 | 6,000 | 20 |
| Tobacco Necrosis | RNA | Single | 0.5×10^6 | 1,500 | 5 |
| Turnip yellow mosaic | RNA | Single | 2×10^6 | 6,000 | 10 |
| Wound tumour virus | RNA | Double | 16×10^6 | 24,000 | 80 |

Adapted after Knight (1974); Sarkar (1969); Hull (1977); Goodman (1977) and Shepherd (1968)

However, there are a few exceptions to such unitary structure. For instance, it has been reported that the double stranded RNA of reovirus occurs in 10 segments (Millward and Graham 1970). Similar reports are also there in respect of wound tumour virus (Kalniakoff *et al.* 1969) and influenza virus (Berry *et al.* 1970).

In order to know about the details of the structural features in these exceptional cases end group analyses have been increasingly done in

recent years. Such analyses are particularly helpful in elucidating the structural alignment of the various segments in such federated nucleic acids.

Primary Structure of Viral Nucleic Acids

Nucleic acids are long chain molecules made up of nucleotides. You all know that the nucleotides are named according to the purine and pyrimidine base they contain. For RNA, the nucleotides are the adenylic, guanylic, uridylic and cytidilic acids. For DNA, these are deoxyadenylic, deoxyguanylic, deoxycytidilic and thymidilic acid. These nucleotides are made up of three simplex components each: phosphate, a sugar (ribose or deoxyribose) and a base (purine or pyrimidine). You also know that these bases are adenine, guanine, cytosine and thymine in DNA and the first three and uracil instead of thymine in RNA. Adenine and guanine are the purine bases and cytosine, thymine and uracil are the pyrimidine bases. Apart from these 5-hydroxymethyl cytosine and 5-hydroxymethyl uracil are also associated with some phage DNA. It is also known that in a double stranded nucleic acid, the two strands are held together by hydrogen bonds between purine and pyrimidine bases. There is, moreover, a specific association between the purine and the pyrimidine bases in that adenine (A) form an association with thymine (T) and guanine (G) associates with cytosine (C). In case of double stranded RNA molecules, as in reovirus, the association is between adenine and uracil (U).

The primary structure of nucleic acid relates to the proportion and arrangement of these various nucleotides in a specific manner. An important step in characterizing the primary structure of nucleic acid would be to determine (1) the proportions of purines and pyrimidines present and (2) the sequence of arrangement of the bases or of the nucleotides they are parts of. Since it is well known that the kind and the sequential arrangement of the nucleotides determine the ultimate genetic function of the nucleic acids, their elucidation would naturally go a long way in establishing the biological nature of viral nucleic acids.

Determination of Base Ratios and Nucleotide Proportion

Various methods, both chemical and physical have been used to determine the base ratios of viral nucleic acids. As has been discussed earlier (See Chapter Two) Molar base or nucleotide ratio can be determined by segregating the different bases or nucleotide by hydrolysing the viral nucleic acid under controlled conditions. The nucleotides can then be identified and separated quantitatively by chromatography

(paper, column etc.). Physical characteristics like buoyant density and thermal denaturation profiles of nucleic acids are known to be functions of their molar GC (Guanine+Cytosine) content. Suitable methods for determining the molar base ratios from these parameters have also been standardised. Tables 3.5 and 3.6 list the molar base ratios of some deoxy viruses and ribo viruses respectively.

TABLE 3.5. NUCLEIC ACID BASE RATIOS OF SOME DEOXY VIRUSES
(Moles per cent)

| <i>Virus</i> | <i>Adenine</i> | <i>Guanine</i> | <i>Cytidine/ Hydroxy Methyl Cytosine</i> | <i>Thymine</i> | <i>AT/GC</i> |
|-----------------------------------|----------------|----------------|--|----------------|--------------|
| Adenovirus | 21 | 29 | 29 | 21 | 0.73 |
| Coliphage $\phi \times 174$ | 24 | 25 | 19 | 32 | 1.27 |
| Coliphage T ₄ | 33 | 17 | 17 | 33 | 1.92 |
| Coliphage Lambda | 26 | 24 | 24 | 26 | 1.04 |
| Herpes Simple | 16 | 34 | 34 | 16 | 0.47 |
| Polyoma | 26 | 24 | 24 | 26 | 1.04 |
| <i>Salmonella</i> P ₁₁ | 25 | 25 | 25 | 25 | 1.00 |
| Shope Papilloma | 26 | 24 | 24 | 26 | 1.04 |
| Simian Virus 40 | 26 | 24 | 24 | 26 | 1.04 |
| <i>Tipula</i> Iridescent | 34 | 16 | 16 | 34 | 2.12 |

Adapted from Knight (1974).

TABLE 3.6. NUCLEIC ACID BASE RATIOS OF SOME RIBO VIRUSES
(Moles per cent)

| <i>Virus</i> | <i>Adenine</i> | <i>Guanine</i> | <i>Cytosine</i> | <i>Uracil</i> | <i>AU/GC</i> |
|---------------------------|----------------|----------------|-----------------|---------------|--------------|
| Broadbean mottle | 27 | 25 | 19 | 29 | 1.27 |
| Brome mosaic | 27 | 28 | 21 | 24 | 1.04 |
| Coliphage M ₁₃ | 23 | 26 | 26 | 25 | 0.93 |
| Foot and mouth disease | 26 | 24 | 28 | 22 | 0.93 |
| Influenza | 23 | 20 | 24 | 33 | 0.80 |
| Mouse encephalitis | 25 | 24 | 24 | 27 | 1.05 |
| Poliomyelitis | 29 | 24 | 22 | 25 | 1.15 |
| Reo | 28 | 22 | 22 | 28 | 1.27 |
| Tobacco mosaic | 28 | 24 | 22 | 26 | 1.15 |
| Tobacco necrosis | 28 | 26 | 22 | 24 | 1.05 |
| Tomato bushy stunt | 25 | 28 | 21 | 26 | 1.04 |
| Wound tumour | 31 | 19 | 19 | 31 | 1.63 |

Adapted after Knight (1974).

An analysis of the molar base ratios of the viral nucleic acids reveals that:

(1) there is a great variation in the base ratios of nucleic acids, both RNA and DNA, obtained from different viruses. This is clearly reflected in AT/GC or AU/GC ratios.

(2) In double stranded nucleic acid molecules there is direct one to one correspondence between the purine and the pyrimidine bases. This is evident from the data obtained on nucleic acid from all the DNA containing viruses except $\phi \times 174$ which has single stranded DNA. Similarly, there is such a correspondence in the cases of only two RNA containing viruses, wound tumour and reo, both of which are double stranded RNA. In the other cases, there is no such correspondence. As a result there is lopsided proportions of certain bases. For instance, a higher proportion of uracil than other bases is found in influenza virus.

(3) Similar viruses may have almost identical base ratios. For instance Shope papilloma and polyoma virus and Simian virus 40 all of which are oncogenic and possess nucleic acids with similar base ratios.

(4) Dissimilar viruses may also have almost identical base ratio, for instance Coliphage T_4 and Tipula iridescent virus, both having similar GC content.

Nucleotide Sequence Analyses

We have seen that base ratio analyses do not allow a close insight into the primary structure of nucleic acid molecules. A better approach would be to know about the sequence of arrangement of nucleotides in a molecule. In recent years attempts are being made to elucidate the nucleotide sequences of several viral nucleic acids.

The basic approach is somewhat as follows. The long chain nucleic acid or polynucleotide molecules are partially but specifically degraded into smaller fragments of various sizes. Such degradation is achieved by using specific nuclease enzymes capable of splitting up long chain polynucleotides at specific sites. The enzymes used include endonucleases, exonucleases and restriction endonucleases. These smaller fragments are then purified and separated from one another by gel electrophoresis. After purification, the sequences of nucleotides in these smaller fragments can be determined by further enzymatic degradation followed by their separation and identification with their specific products. By using enzymes of different specification, polynucleotide sequences that overlap, or sort of dovetail, can be obtained. Thus, progressively more and more smaller nucleotide segments can be arranged in their correct order in larger segments of nucleic acid until the correct sequence is deduced.

In recent years sequence analyses have been attempted both on viral RNA and on viral DNA. However, the most complete sequence analysis has been obtained only recently. In 1976 the first sequence analysis of a viral RNA genome that of Coliphage MSZ, was reported (Fiers *et al.* 1976). More spectacular and impressive has been the elucidation of the sequence of $\phi \times 174$ DNA by Prof. Frederic Sanger and his associates (Sanger *et al.* 1977).

In this report Prof. Sanger and his team have established that this viral genome, which is a single stranded circular DNA molecule, consists of 5,375 nucleotides. This is shown to code for nine proteins from nine regions in the genome corresponding to nine genes. The order of these genes, as determined by genetical studies is *A-B-C-D-E-J-F-G-H* (Benbow *et al.* 1975). The protein products of these genes have specific functions in the physiology of $\phi \times 174$. The amino acid sequence of these proteins and the nucleotide sequence of these genes have been directly correlated. Table 3.7 summarises the coding capacity of $\phi \times 174$ nucleic acid.

TABLE 3.7. CODING CAPACITY OF $\phi \times 174$ GENOME

| Gene | Number of nucleotides | Codes for protein (enzyme) needed in | Mol. wt. of protein calculated from sequence | Mol. wt. of protein based on SOS gel electrophoresis |
|------------------|-----------------------|--|--|--|
| A | 1536 | Double stranded viral nucleic acid synthesis | 56,000 | 55,000-67,000 |
| *B | (360) | Viral single stranded DNA synthesis | 19,000 | 13,845 |
| C | 257 | Viral single stranded DNA synthesis | — | 7,000 |
| D | 456 | Viral single stranded DNA synthesis | 16,811 | 14,500 |
| *E | (273) | Lysis of Host | 9,940 | 10,000 |
| J | 114 | A small basic protein | 4,097 | 5,000 |
| F | 1275 | Capsid protein | 46,400 | 48,000 |
| G | 525 | Capsid protein | 14,053 | 14,000 |
| H | 984 | Capsid protein | 35,800 | 37,000 |
| Non-coding Total | 228 | | | |
| | 5375 | | | |

*B and E are included genes. After Sanger *et al.* (1977).

Other interesting features noted are:

(1) The capacity of the same stretch of DNA to code for two proteins which are translated in different reading frames.

(2) Position of certain genes are within the region of another gene *viz.* Gene *E* is contained in gene *D* and gene *B* in gene *A*.

(3) Presence of sequences responsible for promotion and termination of gene functions.

It should be noted that sequencing is a difficult and time consuming task, necessitating the utilization of a large number of sophisticated techniques. Therefore, before or even while attempting sequencing other methods are used for comparing sequences without actually 'sequencing' them. Two of such procedures are (1) nearest neighbour frequency analysis and (2) homology analysis.

Nearest Neighbour Analysis

In a nutshell, nearest neighbour analysis provides information on the frequencies with which various nucleotide pairs or 'doublets' occur in a particular nucleic acid or polynucleotide molecule. Since four types of nucleotides go to make up a nucleic acid molecule, (4)² or 16 nucleotide pairs can possibly be formed. It is possible to compare nucleic acids in terms of the frequencies with which the various nucleotide pairs occur in them.

Supposing the nearest neighbour analysis of a particular type of nucleic acid is to be done. This nucleic acid is used as a template for the synthesis of fresh polynucleotide sequences using nucleotides containing radioactive phosphorus. Four sets of synthesis are carried out according to the following protocol. In each reaction set only one nucleotide is allowed to be radioactive.

Reaction 1 : $\overset{*}{\text{PPPA}}$, PPPG , PPPC , PPPT + template + polymerase

Reaction 2 : PPPA , $\overset{*}{\text{PPPG}}$, PPPC , PPPT + template + polymerase

Reaction 3 : PPPA , PPPG , $\overset{*}{\text{PPPC}}$, PPPT + template + polymerase

Reaction 4 : PPPA , PPPG , PPPC , $\overset{*}{\text{PPPT}}$ + template + polymerase

The polymerase enzyme repeatedly joins the nucleotides forming a polynucleotide strand complementary to the template. The newly synthesised strands are separated and purified by suitable means. These are then enzymatically digested such that only nucleotide monophosphates are released. The entire digestion is so specific and operated in such a manner that the radioactive phosphorus attached to one

nucleotide prior to degradation gets attached to the neighbouring nucleotide on degradation (Fig. 3.1).

The degradation product are then separated by paper chromatographically and identified. The radioactivity, if any, of each of them is then determined. From this data the relative frequency of occurrence of the dinucleotide pairs or 'doublets' can be determined.

Supposing in reaction (1), guanosine derivative present gets associated with the radioactive adenosine derivative more frequently than do the other nucleotide derivati e during the synthesis of complementary strands. In that case, on enzymatic degradation guanosine monophosphate will have more radioactivity than will have the other nucleotide monophosphates *viz.* adenosine monophosphate or thymidine monophosphate or cytosine monophosphate. The nearest neighbour frequencies of different nucleic acids can then be analysed and compared. The 'doublet' frequencies for some viral DNAs are given in Table 3.8.

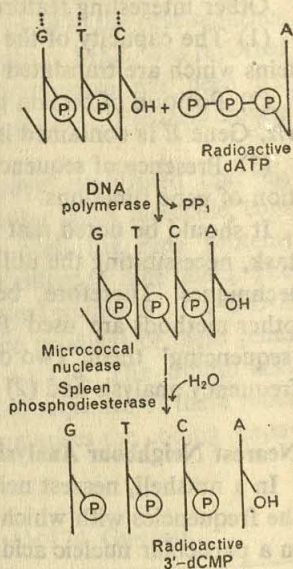


FIG. 3.1. Method for determining nearest neighbour frequencies in DNA (see text).

TABLE 3.8. DOUBLET FREQUENCIES OF SOME VIRAL DNAs
(per cent)

| Virus | Poly- Nucleo- oma | <i>T₁</i> coliphage | Shope Papil- loma | <i>T₂</i> | Vaccinia | Herpes | Phage | Coli- phage <i>T₄</i> | Coliphage $\phi \times 174$ |
|-------|-------------------------|-----------------------------------|-------------------------|----------------------|----------|--------|-------|--|--------------------------------|
| ApT | 5.6 | 6.4 | 5.7 | 6.5 | 6.9 | 6.8 | 7.0 | 6.4 | 5.9 |
| TpA | 5.0 | 4.8 | 4.5 | 6.2 | 5.8 | 6.8 | 4.7 | 5.5 | 4.6 |
| GpC | 6.4 | 7.6 | 7.0 | 7.8 | 5.4 | 6.5 | 7.2 | 7.0 | 8.0 |
| CpG | 2.2 | 7.0 | 3.0 | 5.8 | 6.5 | 6.5 | 7.0 | 5.3 | 5.9 |

(Modified after Josse *et al.* 1961).

An analysis of these results reveals that nearest neighbour frequencies in nucleotide pairs (doublets) of *T* series of bacteriophages are remarkably similar. According to Josse, the degree of similarity is even more than would be expected on chemical, morphological or serological grounds. A similar resemblance is also discernible between the doublet frequencies of polyoma and shope papilloma viruses and

that of herpes and vaccinia viruses. However, there are marked differences in between these groups. Nearest neighbour analyses have also been made on RNA viruses, including those of tobacco mosaic and turnip yellow mosaic viruses.

On the whole it could be said that comparison of nearest neighbour frequencies can be useful in assessing phylogenetic similarities between different viruses. However, they are no way a substitute for total sequence analysis.

Homology Analyses by Hybridization

Another method for gaining information about nucleic acid sequences is based on hybridization of nucleic acid molecules. The basic procedure is as follows:

The two strands of a double stranded nucleic acid are associated together mainly by hydrogen bonds between complementary bases (A : T or U and G : C) of the two strands. The two strands could be separated by heating which breaks up the hydrogen bonds. Separation could be achieved by chemical means also. Separation of nucleic acid strand is called its denaturation. When this is achieved by heating, the process is also referred to as molecular melting.

When heat separated nucleic acid strands are allowed to cool gradually, they tend to reassociate. This phenomenon is called renaturation or annealing of strands. If denatured strands of a second species of nucleic acid are added to those of originally denatured one during the annealing process, strands of the second nucleic acid may compete with the original ones in reforming the double stranded structure. This would occur only if substantial nucleotide sequences of both the species are the same or very similar. Such similarity or homology would lead to formation of hybrids between complementary strands of the two nucleic acids. Therefore, the procedure is known as *hybridization*.

Experimentally, the original strands are immobilized after separation on agar-gels or on nitrocellulose films. The strands of the second nucleic acid species are similarly denatured and broken or sheared into smaller fragments. The second species is made radioactively labelled previously. The broken radioactive strands are then made to anneal with the immobilized original strands. After a suitable period of incubation the excess or unassociated nucleic acid fragments are washed off. The immobilized DNA of the original species are then analysed for radioactivity. More the homology, more will be the association as reflected in higher radioactivity in the immobilized nucleic acid strands.

Hybridization experiments essentially give us information regarding the homology existing between two species of nucleic acids. This information have been used in

(1) evaluation of the degree of similarity of the nucleic acid from different or similar types of viruses.

(2) investigation of the amount of replicating viral nucleic acid present at different times after infection.

(3) estimation of the viral genome incorporated into host nucleic acid.

(4) determination of the presence or absence of virus specific messenger RNA, and

(5) discrimination between types of the RNA present at various stages after infection (*see* Bovre *et al.* 1971; Knight 1974; Chattopadhyay *et al.* 1976).

Secondary Structure of Viral Nucleic Acids

The nucleic acid component of viruses is generally a single linear molecule. A few, like the Simian Virus 40 and polyoma virus have circular molecules. The structure *i.e.* the three dimensional configuration of these molecules assumes importance since these single molecules have to be specifically accommodated in the mature virus particles. These configuration *in vivo* may or may not follow the classical configuration prototype *i.e.* the double helix (Watson and Crick 1953). In fact, there are evidences which indicate that the circular single stranded nucleic acid molecules as in coliphage $\phi \times 174$ remain in a twisted or a supercoiled state. It is reasonable to assume that the biological function of the viral nucleic acid can be directly linked to their secondary configuration (Plate IV).

Various physical and chemical techniques, mentioned earlier, have been used in the elucidation of the secondary structure of nucleic acid. Considerable work has been done to elucidate the secondary structure of the TMV-RNA. It has been shown that the alignment of the RNA molecule within the TMV particle is different from isolated TMV-RNA molecule (Gierer 1960). TMV-RNA in solution is not entirely helical as would be the case while it is packed in a particle. Studies on some other RNA viruses reveal that in some of them, like the reovirus, the RNA genome is fragmented into ten segments (Thaticin *et al.* 1968). Similarly wound tumour virus infecting clover has 12 segments (Reddy and Black 1973). Similar segmentation of viral RNAs have been noted also in influenza virus (Duesberg 1968). Apparently these segments are responsible individually for different functions.

The secondary structure of viral DNAs seem to be more stable in

the sense that they do not show much change when isolated from intact particles. The form essentially is the classical double helix.

PROTEINS

Proteins are one of the basic biochemical units constituting a cell. They are the agents which as enzymes carry out all the work of the cells and bring about the innumerable cellular chemical reactions. They also provide structural elements as in muscles, hairs etc.

It has been well established by Emil Fisher that proteins are composed of twenty odd amino acids linked together in various numbers and combinations. They are linked together covalently by the group —HN—C—O— , which is formed by the basic group —NH_2 of one amino acid with the carboxylic group —COOH of the next amino acid. Thus is formed a peptide chain which naturally has a free H_2N group at one end and a free —COOH group at the other. This chain represents the *Primary Structure* of the proteins. In proteins containing more than one chain there may be interchain covalent linkage through disulphide bonds (—S—S—) between sulphur containing amino acids like cystine.

In some cases, the peptide chain is not wholly straight. In parts it shows helical configuration. This helical configuration, also called the *alpha helix*, is formed due to hydrogen bonding between —CO and —NH group of amino acids situated at regular intervals. The combined straight and helical configuration represent the *Secondary Structure* of a protein.

The peptide chain is often folded. The folded structure represents the *Tertiary Configuration* of the peptide chain.

Often there may be more than one peptide chain or subunit forming a protein structure. The number and spatial relationship of these subunits represents the *Quaternary* structure of the proteins. The tertiary and quaternary structure can be determined by X-ray crystallographic studies (See Kendrew 1959).

Generally, proteins form the outer component of virus particles. In effect, it is the outer protein part or the coat, sometimes also called the sheath, which gives the virus particles their characteristic shape. In most cases the protein coats are not unitary structures, rather are composed of a varying number of identical subunits. The coat has antigenic properties and is mainly protective towards the central nucleic acid against host cell nucleases. It is reported to be resistant against certain proteolytic enzymes as well.

In addition to coat proteins, a few complex viruses contain certain internal proteinaceous entities. These have characteristics different

from those of coat proteins. The exact nature and function of these proteins, however, are not clear, though at least a few of them are known to be enzymic. As for example, phage lysozyme (Kozloff 1968) and influenzal neuraminidase (Webster 1969) associated with bacteriophages and influenza virus respectively. In recent years, enzymes involved in nucleic acid synthesis, have also been detected in viruses, as for instance the RNA directed DNA polymerase ('reverse transcriptase') found in Rous Sarcoma virus (Temin and Mizutani 1970). Table 3.9, lists some of the non-coat proteins associated with viruses.

TABLE 3.9. NON-COAT PROTEINS ASSOCIATED WITH VIRUSES

| <i>Non-Coat Proteins</i> | <i>Virus</i> | <i>Reference</i> |
|--------------------------------|------------------------------|----------------------------|
| Virol Lysozme | Bacteriophage T ₂ | Kozloff 1968 |
| Influenzal neuraminidase | Influenza virus | Webster 1969 |
| | | Drzneik 1972 |
| RNA transcriptase | Reovirus | Shatkin and Sipe 1968 |
| RNA polymerase | New Castle disease virus | Kingsbury 1973 |
| RNA directed DNA polymerase | Rous Sarcoma virus | Temin and Mizutani 1970 |
| | Murine Leukemia virus | Baltimore 1970 |

Adapted from Knight (1974).

Primary Structure of Viral Proteins

A prerequisite for the analysis of viral proteins or for that matter any protein, is to obtain a purified preparation. The protein component in viruses have to be separated from the non-protein constituents. This is usually achieved by routine procedures mentioned earlier (See Chapter Two). These include, as you will recall (1) mild alkali treatment, (2) Cold 67 per cent acetic acid precipitation method and (3) the phenol method.

Under controlled conditions, exposure of the virus particles to mild alkali or detergents leads to partial to total release of the particle proteins. These can then be separated, redissolved and reprecipitated to obtain purer preparations. Further treatments like dialysis followed by lyophilization helps in obtaining more purified preparations.

It has been noted that divergent viral types require different preparative procedures to obtain maximum satisfaction. For instance the cold acetic procedure which gives good result with tobacco mosaic virus, is quite unsatisfactory in dealing with turnip yellow mosaic virus. Selection of the right procedure comes with experience and

certain amount of trial and error.

One problem associated with the preparation of viral proteins is the establishment of their homogeneity. Homogeneity is essential, otherwise no proper clue to their primary structure can be obtained. While selective precipitation, such as at the isoelectric point have been attempted with some success, more sophisticated techniques like electrophoresis, gel filtration and density gradient centrifugation are also in use.

In general, all of the methods in use to prepare viral proteins are capable of causing various degrees of degenerative changes. These changes are generally at the level of quaternary configuration of the protein. Some of these changes are reversible. Some, however, are not. In order to distinguish between native and denatured proteins, Anderer (1959) suggested a list of criteria. Taking TMV protein as the model he noted that a native viral protein should be (1) soluble in aqueous solution, (2) aggregate into the parent virus like rods at certain specific pH levels, (3) with appropriate viral nucleic acid should be able to reconstitute into infectious particle and (4) show resemblance to the intact virus particles, in the amount of the specific viral antibody it binds.

Analysis of the Primary Structure

The primary structure of a protein is its basic structure. The secondary, tertiary and quaternary structures are essentially dependent upon the primary features, which in their turn are derived from the composition of the amino acids, and their sequential arrangements.

Amino Acid Composition of Viral Proteins

The amino acid residues of purified viral proteins are usually released by hydrolysis of the protein under controlled conditions. This is achieved by taking 2-5 mg of protein in one ml of 6 N HCl in a sealed glass tube and heating it at 110°C for 36 hours under vacuum. By this method a majority of the constituent amino acids are almost quantitatively liberated. However, while most of the amino acids are little affected by such treatment, a few like threonine, tryptophan and cysteine are destroyed to a considerable extent. Therefore, either these amino acids are separately isolated under specific conditions and assayed or less drastic hydrolytic procedures are adopted (*See Block 1958*).

The amino acids thus liberated can be qualitatively determined by paper chromatography using the conventional technique (Martin and Synge 1941). Precise quantitative analysis can be efficiently done using

automatic amino acid analysers. These analysers are built on the principles of ion exchange chromatography as utilized for amino acid analyses by Moore and Stein (1965). The analyser records the amino acids present in the protein hydrolysate both qualitatively and quantitatively. The relative quantities of amino acids present are indicated by the peak values observed in the recording graph paper. However, in order to know the absolute quantities of amino acids present, it is customary to calibrate the relative values against standards prepared earlier using known quantities of the various amino acids.

Table 3.10 lists the amino acid composition of some viral coat proteins.

TABLE 3.10. AMINO ACID COMPOSITION OF SOME VIRAL COAT PROTEINS
(Relative number per unit)

| <i>Virus Amino Acid</i> | <i>Tobacco Mosaic Virus</i> | <i>Tobacco Necrosis Virus</i> | <i>Cucumber Mosaic Virus</i> | <i>Coliphage fd</i> | <i>Coliphage QB</i> |
|-------------------------|-----------------------------|-------------------------------|------------------------------|---------------------|---------------------|
| Alanine | 14 | 13 | 17 | 9 | 15 |
| Arginine | 11 | 14 | 24 | 0 | 7 |
| Asparagine | 10 | 0 | 0 | 0 | 8 |
| Aspartic Acid | 8 | 18 | 30 | 3 | 7 |
| Cysteine | 1 | 2 | 0 | 0 | 2 |
| Glutamine | 9 | 0 | 0 | 1 | 8 |
| Glutamic Acid | 7 | 20 | 20 | 2 | 5 |
| Glycine | 6 | 8 | 16 | 4 | 7 |
| Histidine | 0 | 1 | 4 | 0 | 0 |
| ISO-Leucine | 9 | 11 | 16 | 4 | 4 |
| Leucine | 12 | 10 | 26 | 2 | 12 |
| Lysine | 2 | 12 | 18 | 5 | 7 |
| Methionine | 0 | 6 | 8 | 1 | 0 |
| Phenylalanine | 8 | 12 | 7 | 3 | 3 |
| Proline | 8 | 15 | 6 | 1 | 8 |
| Serine | 16 | 14 | 24 | 4 | 9 |
| Threonine | 16 | 16 | 10 | 3 | 12 |
| Tryphopin | 3 | 0 | 2 | 1 | 0 |
| Tyrosin | 4 | 11 | 4 | 2 | 4 |
| Valine | 14 | 14 | 7 | 4 | 13 |

Taken from Knight *et al.* (1974), Konigsberg *et al.* (1970); Van Regenmortel (1967); Anderer *et al.* (1960); Clyemote & Grogan (1969).

A survey of the amino acid composition data reveals that viral coat proteins are constituted of the common protein amino acids and there is no evidence of any unusual amino acid, like diaminopimelic acid which is found in bacteria. Studies also indicate that the amino acids are all *L*-isomers, the form which is universally found in other living forms.

Molar distribution of amino acids in viral protein can be calculated from its molecular weight. The molecular weight is usually determined by any convenient method. This value divided by minimal molecular weight (the summation of the relative number of the individual amino acid) gives a figure whose nearest whole number would be the factor by which the relative number of individual amino acid residues must be multiplied to give the actual number per protein molecule.

To illustrate the point we might survey the amino acid analysis of TMV Coat protein. Data (Table 3.10) reveal that the minimal molecular weight *i.e.* the summation of the relative number of amino acids per mole is 158. Molecular weight of TMV Coat protein subunit is calculated independently to be 17,500. Therefore, the factor *i.e.* the nearest whole number ratio between the actual molecular weight and the minimal molecular weight is about 118. Therefore, the relative number of individual amino acid must be multiplied by 118 to give their actual number per mole.

End Group Analyses and Protein Subunits

The amino acid composition, though very useful does not tell us anything about the protein substructure. For instance, it does not throw much light on the number of subunits in a protein or on the number of chains in a protein or its subunits. To obtain information on these, other parameters, like the number of endgroups are of immense help.

You all know that a protein or peptide chain consists of a linear sequence of amino acid residues with two free ends. One of these ends has a free-NH₂ (amino) group and is called the N-terminal end. The other has a free-COOH (Carboxylic) group and is called the C-terminal end. In the usual manner of writing linear formulae for such structures, the amino acid, residue on the extreme left is called the amino or N-terminal residue and the residue on the extreme right is called the carboxyl or C-terminal residue. These terminal residues could be identified easily if they could be selectively cleaved and separated from the main protein or peptide chain.

In recent years an easy and effective enzymatic tool has been developed to achieve this. A group of enzymes called the *carboxypeptidases* have been found to be capable of selectively cleaving C-terminal amino acid residues from protein units (See Ambler 1972). There are several types of carboxypeptidases, namely carboxypeptidases A, B, C, D and E. All of them are not capable of cleaving all the C-terminal amino acid residues. However, in combination they can remove most

of them.

The number of C-terminal residues would be a sure guide to the subunits or the chain number in a protein unit. As early as in 1955, Harris & Knight reported that 2320 threonine residues were liberated per mole of TMV when the virus was treated with carboxypeptidase A. It was suggested by them that there were as many polypeptide chains in the TMV protein. This finding was subsequently confirmed by other reports (Franklin *et al.* 1957).

In the above study only threonine residues were liberated. This indicated that all the polypeptide chains or subunits which form the TMV coat protein are identical in their amino acid composition, and probably in amino acid sequence as well.

There are a few examples where it is known that the viral protein component is more complex and is composed of dissimilar subunits. T-even coliphages are a case in point. It has been demonstrated that the head protein in these phages is of different composition from the tail sheath protein. Therefore, end group analysis revealed two different kinds of amino acid residues (See Mathews 1971).

Carboxypeptidases are not much helpful in the analysis of N (amino) terminal end. There are effective, chemical methods for determining those ends. In one of these methods, the protein is reacted with fluorodinitrobenzene (FDNB) in mildly alkaline solutions. FDNB reacts with the N-terminal residues to give rise to a dinitrophenol (DNP) substituted residue. Upon hydrolysis the DNP group remains associated with the N-terminal amino acid, and can be easily identified.

End group analyses are, therefore important for elucidating the primary structure of proteins, particularly their substructure. This is also helpful in the determination of the molecular weights of the subunits. The values thus obtained for such viruses compared favourably with those obtained from amino acid analyses and electrophoretic data.

Amino Acid Sequence Analysis

For complete understanding of the primary structure, the next step to be adopted would be to analyse the sequence of amino acids. The sequence can be analysed following a pattern similar to one adopted for the determination of nucleic acid sequence analysis. The step-wise approach is somewhat as follows:

- (a) Cleavage of a large polypeptide chain into smaller fragments,
- (b) Determination of the sequence of these fragments, and
- (c) Determination of the sequence of amino acids in the individual fragments.

The first step is accomplished by treating the protein chain with the enzyme trypsin. This enzyme specifically break the bonds next to basic amino acids like arginine and lysine. These amino acids are scattered at random in the polypeptide or protein chains. Therefore, these chains are cleaved at random, retaining at the same time a degree of specificity in that all the fragments contain one basic amino acid at one end.

These fragments or small peptide chains then can be segregated by conventional methods like electrophoresis and chromatography. Using a combined electrophoretic and chromatographic procedure Woody & Knight (1959) were able to successfully analyse the tryptic digests of TMV protein coat.

The next step would be to determine the amino acid sequences of the individual tryptic fragments. This step can be illustrated by the work of Ramachandran and Gish (1959) on TMV coat protein. To achieve this first the N-terminal end of these tryptic fragments or peptides was determined. Subsequently the same peptide was treated with the enzyme leucine amino-peptidase. This enzyme catalyses the hydrolytic cleavage of amino acids in a stepwise fashion from the N-terminal end of the peptide chains. At various time intervals small volumes from this mixture were removed and analysed by DNP method. Thus at successive time intervals smaller and smaller peptides were present in the aliquots. The N-terminal end of these were successively determined by DNP method. In this manner the identity of the cleaved amino acid and consequently the sequence of amino acid were determined.

- | | |
|---|-----|
| Thr.—Val—Val—Gln—Arg (Tryptic fragment) | (1) |
| FDNB treatment | |
| Thr (DNP)—Val—Val—Gln—Arg | (2) |
| Leucine amino peptidase | |
| Thr (DNP)+Val—Val—Gln—Arg | (3) |
| FDNB treatment | |
| Thr (DNP)+Val (DNP)—Val—Gln—Arg | (4) |
| Leucine amino peptidase | |
| Thr (DNP)+Val (DNP)+Val—Gln (DN)—Arg | |
| FDNB treatment | |
| Thr (DNP)+Val (DNP)+Val (DNP)—Gln—Arg | (5) |
| Leucine amino peptidase | |
| Thr (DNP)+Val (DNP)+Val DNP—Gln—Arg | (6) |
| FDNB treatment | |
| Thr (DNP)+Val (DNP)+Val (DNP)+Gln (DNP)—Arg | (7) |
| Leucine amino peptidase | |
| Thr (DNP)+Val (DNP)+Val (DNP)+Gln (DNP)+Arg | (8) |

The next step was to determine the sequence of the tryptic fragments. To achieve this like the normal procedure is to obtain the overlapping sequences or 'bridge peptides' whose sequence would overlap those of the peptides obtained by tryptic digestion.

The 'bridge peptides' were obtained by digesting portions of TMV protein with various proteolytic enzymes like chymotrypsin, pepsin and subtilisin which split the peptide chains at random. The resultant small peptides were separated and more sequences determined as earlier. These sequences were then matched against the sequences of peptides obtained from tryptic digests.

After much painstaking analyses the sequences of the tryptic peptides could be determined. The following will illustrate the procedure.

The sequences of two tryptic digests of TMV Coat protein were found to be the following by Ramachandran and Gish.

Val - Tyn - Arg (Sequence A)

Phe - Pro - Asp - Phe - Asp - phe - Lys (Sequence B)

Chemotryptic digestion of TMV protein yielded a sequence

- Lys - Val - Tyn (I)

Now in the tryptic digest two peptides with a lysine residue were found. These were

- Lys - Pro - Ser - (a)

- Asp - phe - Lys - (b)

In sequence (a) Lysine is followed by proline whereas in sequence (b) Lysine is the last residue. Therefore, the chymotryptic digest (I) is probable to be linked to the tryptic fragment (b) making a sequence

- Asp - phe - Lys - val - Tyn

Now the only sequence with Valine - Tyrosine dipeptide found by Ramachandran and Gish were

Val - tyn - Arg (Sequence A)

Therefore, the probable sequence which can be easily constructed would be

Asp - phe - lys - val - Tyn - Arg (III)

Again the only sequence obtained with a partial sequence of Asp - phe - lys was

Phe - pro - Asp - phe - Asp - pue - lys (Sequence B)

Therefore sequence (B) can be seen to be matched with fragment (III) forming a sequence

Phe - pro - Asp - phe - Asp - phe - cys - val - tyn - Arg.

The various bridge peptides thus bridge up the tryptic sequence (A) and (B) giving a new sequence (C).

Sequence analysis have also been done on the protein coats of such viruses as bacteriophages *fr*, *f₂*, *MS₂*, *fd* and *QB* (See Knight 1974).

It should also be noted that while the sequences are by and large specific for a type of virus, strange variations occur reflecting both addition and deletion in the viral genome.

Secondary and Higher Structures

Once the primary structure is determined the secondary structure and the other higher configurations of the viral protein can be elucidated. These latter studies are made possibly by such physical techniques as X-rays crystallography and sedimentation analyses. The spatial arrangement of the various subunits of the protein coats and their alignment with the central nucleic molecule can be studied by these methods. These have been dealt with in the next chapter (See Chapter Four).

CARBOHYDRATES

Carbohydrates found in viruses can be categorized into two groups depending on their location within the virus particle. In the category would fall the carbohydrates associated with viral nucleic acids, namely, the ribose and the deoxyribose sugars. Either of them is to be found in all the viruses.

The other category would include, what may be called, the non-nucleic acid carbohydrates. These are found mostly associated with animal viruses but are reported from bacterial and plant viruses as well.

The non-nucleic acid carbohydrate moiety may be a simple sugar like glucose or gentiobiose found in T-even coliphages in which they are linked to the hydroxymethyl cytosine residue. They may also be found associated with proteins (glycoproteins) or lipids (glycolipids) as in animal and plant viruses.

Table 3.11 lists the non-nucleic acid carbohydrate components of some viruses.

Detailed analysis of the Glycoprotein and Glycolipid components reveal that the carbohydrates which make up these entities are fucose, galactose, glucosamine and mannose. In some viruses like the Sindbis virus sialic acid has been found. Sialic acid has a role to play in virus haemagglutination.

It has also been revealed that the protein and carbohydrate moieties in glycoproteins are linked by formation of bonds between the carbohydrate chain and asparagine, serine and threonine residues of proteins.

TABLE 3.11. SOME VIRUSES CONTAINING NON-NUCLEIC ACID CARBOHYDRATES

| <i>Virus</i> | <i>Carbohydrate found as</i> |
|--|---|
| Herpes Simplex | Glycoprotein |
| Influenza | Glycoprotein and Glycolipid |
| Murine Leukoemia | Glycoprotein |
| Simian Virus 5 | Glycoprotein and Glycolipid |
| Potato yellow dwarf | Glycoprotein |
| Sindbis virus | Glycoprotein (Sialic acid) |
| Bacteriophage T ₂ /T ₄ | Gentiobiose or glucose (linked to hydroxymethyl cytosine residue of nucleic acid) |

Adapted after Knight (1974).

LIPIDS

Lipids, have been found to be associated with a large number of viruses representing all the major groups. However, they are mostly found associated with animal and bacterial viruses. Only a few plant viruses have lipids as one of the chemical constituents. Table 3.12 lists the lipids associated with some viruses.

It can be seen from Table 3.12 that there are several kinds of lipids extracted from viruses. Phospholipids of different types are found associated with some of them. Cholesterol and triglycerides are also very common. It is interesting to note that none of these components are exclusive to viruses and are very common in nature.

Another point of significance is that all the lipid containing viruses are surrounded by a membranous envelope which is the sole repository of the lipid components. Interestingly, these viruses mature on the host cell membranes during replication and are liberated by budding of the membrane itself. Therefore, it had earlier been suggested that the viral lipids were derived from the host cell membrane and were not viral in the strict sense of the word. However recent studies have revealed that there is significant difference, both qualitative and quantitative, between the lipids extracted from virus cultures and those isolated from the cell membranes of the hosts in which they were grown. For instance, it has been reported that simian virus 5 contains a phospholipid phosphatidyl inositol which is absent in the membranes of monkey kidney cell in which the virus was grown (Klenk and Choppin 1969).

The nature of the chemical alignments of the viral lipids with other components is not very well understood. However, it is possible that

TABLE 3.12. LIPIDS ASSOCIATED WITH SOME VIRUSES

| <i>Virus</i> | <i>Lipids</i> | <i>Per cent Present</i> |
|------------------------------|--|-------------------------|
| Equine encephalomyelitis | Phospholipids; Cholesterol; Triglycerides | 54 |
| Fowl pox | Phospholipids, Cholesterol; Triglycerides of Fatty acids. | 27 |
| Herpes simplex | Phospholipid | 22 |
| Influenza | Phospholipids, Cholesterol, Triglycerides | 19 |
| Potato yellow dwarf | Phospholipids, Sterols | 20 |
| <i>Pseudomonas</i> phage PM2 | Phospholipid | 10 |
| Simian Virus 5 | Phospholipid, Cholesterol, Triglycerides | 20 |
| Tomato spotted wilt | Phospholipids, Uncertain type | 19 |
| Vaccinia | Phospholipids, Cholesterol, Triglycerides. | 5 |

Adapted after Joklik (1966); Best (1968); Vidaver *et al* (1973).

these are linked loosely to the proteins and to polysacchrides where they are present forming lipoprotein and glycolipid complexes.

OTHER CHEMICAL COMPONENTS

In addition to nucleic acids, proteins and minute quantities of carbohydrates and lipids, some other substances are found in highly purified preparations of certain viruses. For instance polyamines are found in significant amounts in both bacteriophages T_2 and T_4 . The principal polyamines reported are putrescine, spermidine and spermine (Ames *et al.* 1960).

Polyamines have also been reported to occur in herpes virus and influenza virus. Traces of polyamines have also been reported in plant viruses like turnip yellow mosaic virus and broad bean mottle virus.

Inorganic metal cations have also been found to be associated with viral protein and nucleic acids. The exact nature and role of such association is not yet clear.

Chapter Four

MORPHOLOGY AND ARCHITECTURE OF VIRUSES

The concept of *contagium vivum fluidum* propounded by Beijerinck during the end of the last century (1898) enunciated that viruses were fluid in their physical nature. In the absence of any evidence to the contrary, the idea, though doubted by a few (Loeffler and Frosch, 1908), received a limited acclaim and acceptance from scientists for some time. Dearth of suitable instruments and techniques, of course, was the main stumbling block in the way of thorough investigation into the physical nature of viruses. A proper assessment was still a far cry and had to await subsequent technological developments.

Three events occurring within a very short span of about five years (1930-1935) paved the way for a radical change in the entire situation. These events were (1) the development of ultra-centrifuge by Svedberg (1925) and its use in isolating viruses (Schlessinger 1933), (2) the development of electron microscope by Knoll and Ruska (1931), and (3) successful chemical precipitation of tobacco mosaic virus particles by Stanley (1935). Conclusive proof in favour of particulate nature of viruses was gradually and convincingly forthcoming in the subsequent years, demolishing even the traces of lingering doubts which might still be there in anybody's mind. The not so well founded conviction of Loeffler and Frosch was at last vindicated.

Availability of virus particles in the pure form coupled with the use of sophisticated techniques like electron microscopy and X-ray crystallography led to the discovery of a new vista showing a tremendous range and wide diversity in the morphology and architecture of viruses. The variety observed was not only restricted to such attributes as shape, size and weight but was also noted in their internal architecture.

One interesting feature to be observed in this context is the precise uniformity in the particles belonging to a virus species. In fact, this uniformity is more even than that observed in the cells of a bacterium. It is this uniformity which ultimately is responsible for the crystallisability of viruses.

SHAPE OF VIRUSES

Shapewise, most viruses would fall in any one of the following categories: (a) *spheroidal*, also known as *cuboidal*, (b) *elongated* or *rodlike* and (c) *mixed* or combination particles with shapes which are an admixture of the first two types. These groups in their turn may be with or without envelops (Lwoff and Tournier 1966). Apart from these possible groups there are a few viruses with shapes which do not fall into any of the above categories, like for instance, the 'brick shaped' pox viruses (Easterbrook 1966). Their shapes may be considered to be exclusive to a certain extent.

Shapes of virus species falling into a particular category may show variation amongst themselves. For instance rod shaped particles could be simple elongate (tobacco rattle virus), coir shaped (tobacco mosaic virus), flexuous (Coliphage fd, potato virus X) or much coiled (vesicular stomatitis virus). Similar variation within spheroidal types are also quite common.

The most widely used and effective method for the determination of shape of virus particles is electron microscopy. Utilising the various techniques involved in this method, particularly the technique of negative staining (Brenner *et al.* 1951), it had been possible to gain an insight not only into the broad morphology but also into the internal arrangements of virus particles (See Horne 1967, Knight 1974).

Physical information based on X-ray crystallography (Brenner *et al.* 1954) and sedimentation profile analyses (Williams *et al.* 1960) and chemical data (Harris and Knight 1953) have been of significant importance in the elucidation of ultra-structure of viruses.

Tables 4.1, 4.2, 4.3, 4.4, and 4.5 give the shapes of virus particles, belonging to the major distinctive groups of viruses, namely, the plant, the animal and the microbial viruses. Such categorization, though somewhat arbitrary does not, however, detract anything from the basic information they provide.

SIZE OF VIRUSES

First authentic report on sizes of viruses is that of Elford (1939). He developed collodion filters of known and uniform pore sizes and was able to determine the approximate size of the infective virus particles. His work showed that there was a wide variation in the size of viruses, though on the whole, preparations of any one virus gave a constant and reproducible figure. In an interesting study Black (1958) compared the sizes of several viruses as determined by filtration on the

TABLE 4.1. SIZES OF VIRUSES (nm) DETERMINED BY ELECTRON MICROSCOPY AND FILTRATION

| <i>Virus</i> | <i>Size by electron microscopy</i> | <i>Average size by filtration</i> |
|--------------------|------------------------------------|-----------------------------------|
| Adeno | 700 | 1500 |
| Distemper | 2250 | 875 |
| Herpes simplex | 1800 | 1250 |
| Influenza | 1050 | 1650 |
| Measles | 1850 | 2250 |
| New castle disease | 2100 | 1125 |

After Black (1958). The two sets of data mostly did not correspond with each other. This could be due to artifact introduced during electron microscopy or due to inherent flexibility in the virus particles themselves. On the whole methods like this were found to be far from satisfactory.

TABLE 4.2. SHARES AND SIZES OF SOME GROUPS OF ANIMAL VIRUSES

| <i>Virus</i> | <i>Shape</i> | <i>Size dimension (nm)</i> | <i>Special feature</i> |
|-----------------|--------------|----------------------------|---|
| Adenoviruses | Spheroidal | 70-90 | With projecting fibres |
| Herpesviruses | Spheroidal | 100-150 | With envelop |
| Papoviruses | Spheroidal | 43-53 | Without envelop |
| Parvoviruses | Spheroidal | 18-22 | Without envelop |
| Pox viruses | Brick shaped | 230 × 300 | Enveloped; Main structure distinguishable into a core and two lateral bodies. |
| Arena viruses | Spheroidal | 50-150 | With envelop |
| Coronaviruses | Spheroidal | 70-120 | With envelop |
| Diplornaviruses | Spheroidal | 60-80 | With envelop |
| Myxoviruses | Spheroidal | 100-350 | With envelop and projecting spikes |
| Oncornaviruses | Spheroidal | 100 | With envelop |
| Picornaviruses | Spheroidal | 20-30 | With envelop |
| Rhabdoviruses | Bullefshaped | 75 × 130-230 | With envelop |
| Togaviruses | Spheroidal | 40-60 | With envelop |

Adapted from Knight 1974.

TABLE 4.3. SHAPES AND SIZES OF SOME GROUPS OF PLANT VIRUSES

| <i>Virus</i> | <i>Shape</i> | <i>Size dimensions (nm)</i> | <i>Special features</i> |
|-------------------------|---------------|------------------------------|--|
| Alfalfa Mosaic | Pleomorphic | 18×18; 18×36 18×48; 18×58 | Aggregation of three butter shaped and one spheroidal particle |
| Beet Yellow | Flexuous rods | 10×1250 | |
| Brome mosaic | Spheroidal | 25 | |
| Carnation latent | Rods | 15×650 | Slight bends |
| Cauliflower mosaic | Spheroidal | 50 | |
| Clover wound tumour | Spheroidal | 70 | |
| Cowpea mosaic | Spheroidal | 28–30 | |
| Cucumber mosaic | Spheroidal | 30 | |
| Dahlia mosaic | Spheroidal | 50 | |
| Lettuce necrotic yellow | Bullet shaped | 66×227 | Enveloped |
| Pea enation mosaic | Spheroidal | 28 | |
| Potato virus X | Rods | 13×480–580 | Flexuous |
| Potato virus Y | Rods | 15×730–790 | Flexuous |
| Potato yellow dwarf | Rod shaped | 50–100×200–300 | With envelop |
| Southern bean mosaic | Spheroidal | 30 | Filamentous |
| Tobacco mosaic | Rod shaped | 18×300 | Cylindrical rod |
| Tobacco necrosis | Spheroidal | 28 | |
| Tobacco ringspot | Spheroidal | 30 | |
| Tomato bushy stunt | Spheroidal | 36 | |
| Tomato spotted wilt | Spheroidal | 70–80 | Enveloped |
| Turnip yellow mosaic | Spheroidal | 30 | |

Adapted after Harrison *et al.* (1971); Finch & King (1966).

TABLE 4.4. SHAPES AND SIZES OF SOME ALGAE AND FUNGAL VIRUSES

| <i>Virus</i> | <i>Shape</i> | <i>Size (nm)</i> | <i>Interesting feature</i> |
|----------------------------------|--------------------------|---------------------------|--|
| <i>Algal viruses</i> | | | |
| Cyanophage LPPI | Spheroidal head and tail | 50×60 by 28–37×5.5–7.5 | Tail tapering |
| Cyanophage 11–1 | Spheroidal head and tail | 55×16 by 110 | |
| Cyanophage SM | Spheroidal | 88 | Tail perhaps Indimentary |
| Cyanophage A–S1 | Spheroidal head | 90×23 by 244 | |
| Cyanophage <i>Oscillatoria</i> 1 | Rod shaped | 19×300 | |
| <i>Oedogonium</i> phage | Spheroidal | 240 | |
| <i>Chorda tormentosa</i> phage | Cuboidal | 170 | Dense core with three surrounding layers |

Fungal phages

| | | |
|---|------------|-------|
| Mushroom viruses 1, 2, 4, 5 | Spheroidal | 25—50 |
| Mushroom virus 3 | Rod shaped | 19×50 |
| Penicillium stoloniferum | Spheroidal | 25—30 |
| virus 1 & 2 | | 40—45 |
| <i>P. chrysogenum</i> virus | Spheroidal | 35 |
| <i>P. notatum</i> virus | Spheroidal | 25 |
| <i>Piricularia oryzae</i> virus | Spheroidal | 25—32 |
| <i>Sclerotium cepivo-</i> <i>rum</i> virus | Spheroidal | 30—45 |
| <i>Ophiobolus graminis</i> virus | Spheroidal | 29 |

Adopted from Holling & Stone (1971); Hollings (1972); Safferman & Morris (1963); Palan and Shilo (1973).

TABLE 4.5. SHAPE AND SIZE OF SOME BACTERIAL VIRUSES

| <i>Virus</i> | <i>Shape</i> | <i>Size</i> | <i>Special feature</i> |
|--|----------------------------------|-----------------------------|-------------------------------|
| <i>Bacteriophage</i> | | | |
| Coliphage E ₁ | Spheroidal head and rodlike tail | 75×110 by 16 | |
| Coliphage fd | Filamentous | 6×100 | |
| Coliphage lambda | Spheroidal head and rodlike tail | 54×150 by 10 | Tail flexuous |
| Coliphage T ₂ , T ₄ , T ₆ | Spheroidal head and rodlike tail | 65×95 by 25×110 | |
| Coliphage T ₁ , T ₃ , T ₅ | Spheroidal head and rodlike tail | 50×60×10 by 15 65×170×10 | Tail flexuous |
| Coliphage S ₁₃ | Spheroidal | 27 | |
| Coliphage φ×174 | Spheroidal | 27 | Apical knob; tailless |
| Coliphage QB | Spheroidal | 24 | Without Apical knob; tailless |
| <i>Lactobacillus</i> phage 206 | Spheroidal | 72×138 by 16 | |
| <i>Myxococcus Xanthus</i> phage M×1 | Spheroidal | 75×100 by 15 | |
| <i>Pseudomonas</i> Pc | Spheroidal | 65×160 by 10 | |
| <i>Pseudomonas</i> Pf | Filamentous | 6×1300 | |
| <i>Salmonella</i> If2 | Filamentous | 6×1300 | |
| <i>Staphylococcus</i> 6 | Spheroidal | 4×92×10 by 300 | |
| <i>Phodospirillum</i> spheroides phage RQ 6 P | Spheroidal head and rodlike tail | 65×12 by 250 | Tail flexuous |

Adapted after Bradley & Kay (1960); Knight (1974); Pemberton & Tucker (1977)

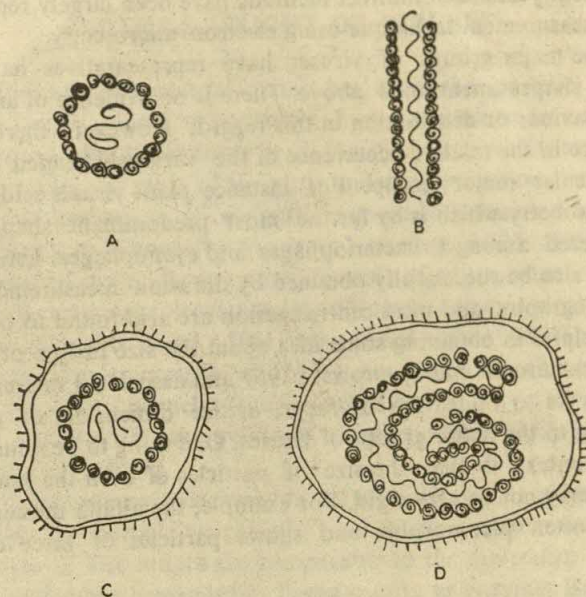


FIG. 4.1. Diagrammatic representation of the arrangement of different components forming a mature virus particle. There are four arrangements namely, (A) naked helical, (B) naked icosahedral, (C) enveloped helical, and (D) enveloped icosahedral. Note the arrangement of the capsid and the nucleic acid.

one hand and by electron microscopy on the other. The size determined by filtration was the mean of pore diameter which retained and of that which passed, the virus (Table 4.6).

TABLE 4.6. SHAPES AND SIZES OF SOME INSECT VIRUSES

| Virus | Shape | Size dimension (nm) | Special feature |
|----------------------------------|---------------|---------------------|---|
| Beetle viruses | Oval | 250×370 | Occur in ovoid inclusions |
| Granulosis viruses | Rod like | 40–62× 270–412 | Occur in inclusion bodies called granules |
| Iridescent viruses | Spheroidal | 150 | No envelop |
| Insect pox viruses | Brick shaped | 250×400 | With core and lateral bodies |
| Polyhedrosis viruses | Rod like | 18–62× 280–332 | Occur in polyhedral inclusion bodies |
| Sac brood virus of bees | Spheroidal | 28 | — |
| Sigma virus of <i>Drosophila</i> | Bullet shaped | 70– 200 | Enveloped |

Adapted after Smith 1967; Knudson 1973; Vagor Bergoin 1968.

The list does not include the dual host viruses like arboviruses which have been listed in the Table.

In recent years such indirect methods have been largely replaced by direct measurement technique using electron microscopy.

All the major groups of viruses have representatives having the various shapes mentioned above. There is no evidence of any exclusive behaviour or distribution in this regard. However, there may be difference in the relative occurrence of the variously shaped particles in particular major group. For instance plant viruses seldom have binal symmetry which is by far the most predominant shape to be encountered amongst bacteriophages and cyanophages. Estimates of size can also be successfully obtained by diffusion measurements, gel chromatography and ultra-centrifugation are also found to be increasingly helpful in obtaining some idea about the size ranges of viruses (see Maramarosch and Koprowski 1967 and associated references).

Tables 4.1 to 4.6 list the size ranges or size dimension of particles belonging to the major groups of viruses. One thing to be remembered in this context is that the size of particles of even the same virus species may not be very rigid. For example, the alfalfa mosaic virus, which is often pleomorphic and shows particles of three different sizes.

TABLE 4.7. NUMBER OF SUBUNITS FORMING CAPSIDS OF SOME ISOMETRIC VIRUSES

| <i>Viruses</i> | <i>No. of Units (Capsomers)</i> | <i>No. of subunits forming Capsomes</i> | <i>No. of Pentamers and Hexamers</i> |
|-----------------------------|-------------------------------------|---|--|
| Coliphage $\phi \times 174$ | 12 | 60 | 12 pentamers |
| Broad bean mottle | 32 | 180 | 12 pentamers 20 hexamers |
| Tobacco ring spot | 42 | 240 | 12 pentamers 30 hexamers |
| Simian virus 40 | 72 | 420 | 12 pentamers 60 hexamers |
| Wound tumour | 92 | 540 | 12 pentamers 80 hexamers |
| Herpes Simplex | 162 | 960 | 12 pentamers 150 hexamers |
| Adenovirus | 252 | 1500 | 12 pentamers 240 hexamers |
| Tipula iridescent | 812 | 4860 | 12 pentamers 800 hexamers |

Adapted after Knight 1974.

PARTICLE WEIGHT OF VIRUSES

Another physical parameter of interest has been the particle weight of a virus. These values have mostly been determined by analytical ultra-centrifugation (cf. Williams *et al.* 1960) which gives the molecular weight or molecular mass of the virus under investigation. From this value the absolute weight of one particle could be calculated by dividing the value taken in grams by Avagadro's number (6.023×10^{23} , which is the number of molecules in one gram mole of any substance). Thus the molecular mass or molecular weight of tobacco mosaic virus (TMV) has been calculated to be 40×10^6 (Harris and Knight 1955). Therefore, one gram molecular weight of TMV is equivalent to 40×10^6 gms. Since the number of particles (molecules) in one gram mole of any substance is 6.23×10^{23} , the weight of one particle can easily be calculated to be 6.68×10^{-17} g.

Table 4.8 lists the particle weights of some viruses. It is interesting to note that the particle weight of a virus or its molecular weight are extremely small. The ranges are comparable to the molecular weights of some cellular biochemicals like haemocyanin or enzymes like glutamate dehydrogenase (Waterson 1968).

TABLE 4.8. PARTICLE WEIGHT OF SOME VIRUSES

| <i>Virus</i> | <i>Mol. weight of virus</i> | <i>Weight of one particle</i> |
|---|---------------------------------|-----------------------------------|
| Adenovirus | 6.7×10^7 | 11.06×10^{-17} g |
| Coliphage fd | 1.1×10^7 | 1.84×10^{-17} g |
| Coliphage fr | 4.1×10^6 | 0.665×10^{-17} g |
| Coliphage $\phi \times 174$ | 7.8×10^5 | 1.29×10^{-17} g |
| Coliphage T ₂ , T ₁ | 24×10^7 | 39.84×10^{-17} g |
| Foot and Mouth Disease | 5.0×10^6 | 0.82×10^{-17} g |
| Polio virus | 5.5×10^5 | 0.88×10^{-17} g |
| Polyoma virus | 2.3×10^7 | 3.7×10^{-17} g |
| Reo virus | 5.8×10^7 | 9.75×10^{-17} g |
| Satellite tobacco necrosis | 4.0×10^5 | 0.664×10^{-17} g |
| Tobacco mosaic | 40×10^6 | 6.64×10^{-17} g |
| Cowpea Chlorotic mosaic | 4.6×10^5 | 7.4×10^{-17} g |
| Cauliflower mosaic | 30×10^7 | 5.0×10^{-17} g |
| Cyanophage, LPPI | 51×10^5 | 8.21×10^{-17} g |
| Vaccinia | 1.33×10^8 | 221.0×10^{-17} g |

Calculated on the basis of molecular mass (weight) and Avogadro's number (6.023×10^{23}).

Another obvious observation is the very wide variation, sometimes three hundred and twenty folds as between vaccinia and coliphage fd

particles, found in viruses. The exact significance of such variation is not clear. Perhaps there is not any. However, it could be that size variation indicate their independent origin from different sources.

SUBUNIT STRUCTURE

A fundamental feature concerning the morphology of viruses had gradually but surely been emerging during the last two decades. It is that a virus particle is in most instances composed of numerous identical protein subunits and one or rarely a few molecules of nucleic acid. It is also obvious that the shape of a virus particle is determined by the protein component. This is because, first, protein is the external viral component and secondly, it comprises the bulk of the particles. Therefore, it is clear that the configuration of and interactions between protein subunits ultimately decide the shape or form of the protein structure and consequently that of the virus particle. (Fig. 4.2)

The fact that such nucleoprotein particles as tobacco mosaic virus (TMV) could be crystallized and apparently had very high molecular weight led to the suspicion as to whether they were single unitary structures or made up of still smaller units. They were found to be too large to be considered as simple macromolecules. Dorothy Hodgkin (1949) was one of the first to point this out experimentally. On the basis of X-ray crystallographic analyses of various proteins, she suggested that in small spherical plant viruses, the protein was a composite macromolecule formed of smaller and identical subunits. Crane (1950) discussing the principles of biological growth predicted that any natural rod shaped object, like the TMV, would be found to be built of a number of similar or even identical subunits arranged in a helix.

In the subsequent years this conception regarding the structure of viruses got confirmed on both theoretical and experimental grounds. In an illuminating summary Francis Crick and James Watson pointed out that the very low nucleic acid content of some of the smaller viruses could not possibly account for the coding of the large peptide chains required for the formation of the protein component, assuming the latter to be a single large entity. This is because the nucleic acid component in them is too small. Also because there would be no space in one virus particle for the amount of nucleic acid necessary for coding such large proteins molecules (assuming that three nucleotides are necessary for coding one amino acid) (Watson and Crick 1956; Crick and Watson 1957).

Crick and Watson further pointed out that if identical protein subunits are used to constitute the protein coat, then it would be

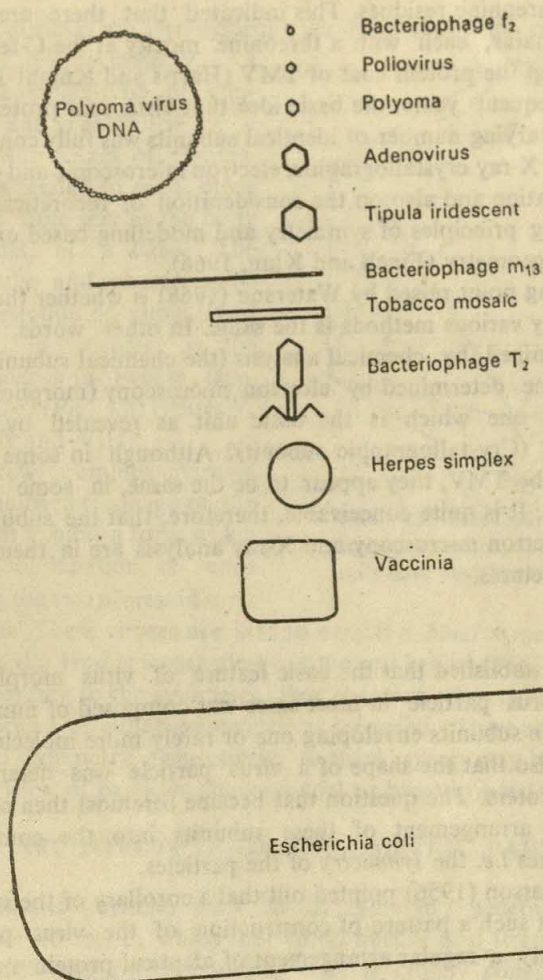


FIG. 4.2. Comparative sizes of some viruses. Their relative sizes in respect of that of the bacterium *E. coli* is also shown. The length of a DNA chain is comparatively much longer than the corresponding virus as depicted in the case of polyoma virus.

possible for the small nucleic acid to code for the small subunit a number of times till the protein structure is built entirely.

Chemical investigations by C.A. Knight and his associates on TMV coat protein confirmed the above contentions. Using the enzyme carboxypeptidase (which can selectively cleave out only the C-terminal amino acid residues) they showed that one mole of TMV particles

yielded 2320 threonine residues. This indicated that there are 2320 polypeptide chains, each with a threonine moiety at the C-terminal end, building up the protein coat of TMV (Harris and Knight 1955).

In the subsequent years, the basic idea that virus coat proteins are made up of a varying number of identical subunits was fully confirmed on the bases of X-ray crystallographic, electron microscopic and chemical experimentation and also on the consideration of theoretical concepts regarding principles of symmetry and modelling based on principles of solid geometry (Finch and Klug, 1966).

An interesting point raised by Waterson (1968) is whether the subunit revealed by various methods is the same. In other words, is the subunit determined by chemical analysis (the chemical subunit) the same as the one determined by electron microscopy (morphological subunit) or the one which is the basic unit as revealed by X-ray crystallography (Crystallographic subunit)? Although in some cases, for example the TMV, they appear to be the same, in some others they are not so. It is quite conceivable, therefore, that the subunits as revealed by electron microscopy and X-ray analysis are in themselves composite structures.

Symmetry

It was thus established that the basic feature of virus morphology was that a virus particle in most cases was composed of numerous identical protein subunits enveloping one or rarely more molecules of nucleic acid. Also that the shape of a virus particle was determined by the virus protein. The question that became foremost then concerned the design arrangement of these subunits into the composite protein structures *i.e.* the *symmetry* of the particles.

Crick and Watson (1956) pointed out that a corollary of the subunit structure is that such a pattern of construction of the virus particle can permit only a regular arrangement of identical protein units. In other words just as the subunits are themselves identical with each other, the environment made up by its neighbours for one subunit should be identical with that of others. They noted that such a situation would create and impose a condition satisfied only by a symmetric arrangement of identical objects about an axis or plane.

A combination of data obtained from various physical and chemical investigations on a large number of plant, animal and microbial viruses led to the conclusion that two basic types of symmetry are generally utilized in the construction of virus particles from protein subunits: the *helical* and the *cubic*. (See Horne and Wildy 1961).

Helical symmetry is exemplified by a spiral staircase in which the

identical rungs are systematically arranged about its central pole or axis. Cubic symmetry is a more complex type of symmetry and is exhibited by a body whose axes of symmetry are related to each other in the same way as the body diagonal of a cube. The particular and characteristic manifestation of cubic symmetry in viruses is the *Icosahedron* (a twenty faceted cube) (Fig. 4.3) (Plates V, VI, VII and VIII).

A few complex viruses like the bacteriophage of the T series exhibit a form which is a combination of units having the two aforesaid symmetries. These viruses are said to exhibit a *binal* symmetry. In these viruses the *head* is icosahedral but the *tail* helical (Fig. 4.4).

According to the symmetry they possess, the virus particles are categorized as either *anisometric* or *isometric*. These show helical and cubical symmetry respectively. A third group may be added to this comprising of particles showing dual or binal symmetry.

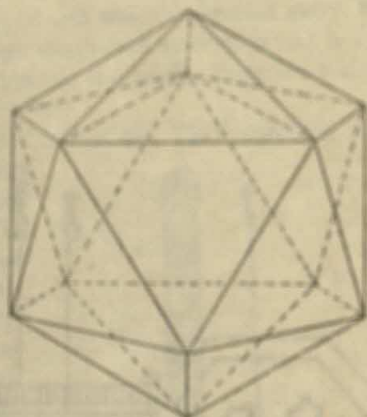


FIG. 4.3. Icosahedron. A twenty faceted structure. Cuboidal virus crystals have such twenty faceted design.

ARCHITECTURE OF ANISOMETRIC PARTICLES

Anisometric particles with helical symmetry generally consist of elongate forms in which the central nucleic acid strand is coiled like a helical spring. The protein coat is closely coiled about its axis, arranged in a succession of rings (Fig. 4.5). In other words, it may be said that in helical viruses the nucleic acid is located in a helical groove on inside of the cylindrical protein coat.

This orderly relation between the nucleic acid and the protein sheath has been beautifully elaborated in the well known plant virus, the tobacco mosaic virus (TMV). These particles are rod shaped structures with the subunits of protein arranged in a helical pattern. It has been calculated that there are about 134 turns to one TMV protein coat. Utilising X-ray diffraction analyses it has been shown that there are 2130 subunits forming the protein coat of TMV (Franklin *et al.* 1959; Casper and Klug 1960). Detailed analysis has shown

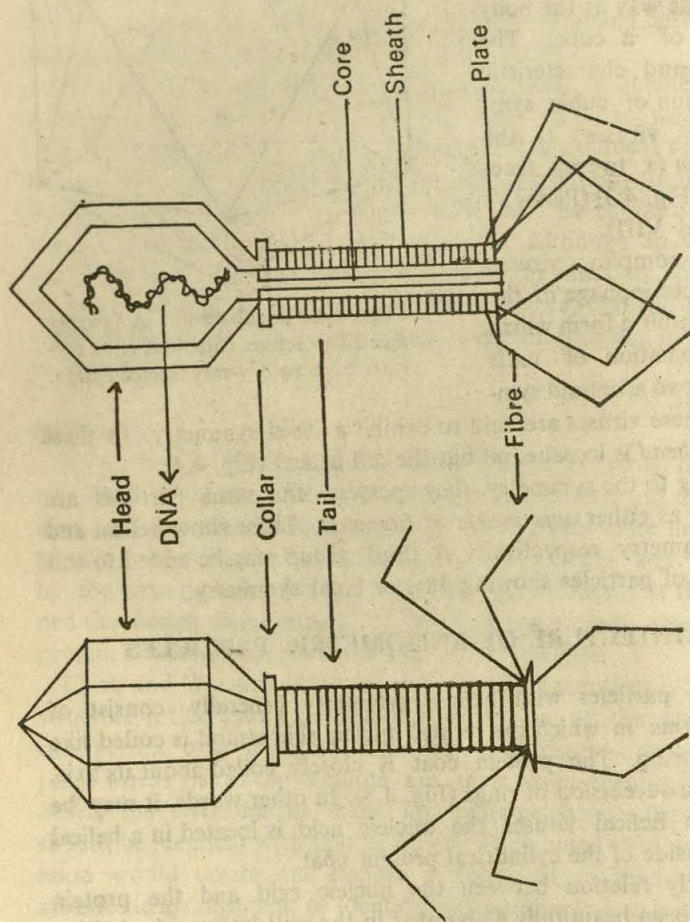


FIG 4.4. Structure of a Bacteriophage. Note the alignment of the head and the tail. The tail fibers are flexible and are attached at the bottom of the tail. Note the position of the collar and the base plate. In 'T' even series of bacteriophages, the plate is hexagonal with the fibers coming out of each corner of the hexagon. Cross-sectional diagram shows the position of the nucleic acid and also the number of spirals in the tail helix (diagram on the right).

that there are 49 subunits distributed over three turns. In an average, these three turns form one structural unit which is repeated every 6.9 nm in the axial direction. In other words each succeeding ring is at a distance of 2.3 nm in the same direction (*i.e.* pitch). There is a central core, about 4 nm in diameter (Fig. 4.6).

RNA, which forms about six per cent of the TMV particles, is a single strand surrounded by the

protein subunits. It has been shown that the RNA strand is arranged according to the turns of the protein sheath subunits. However, the RNA molecule is not located in the central hollow core. Rather it is deeply embedded amongst the successive protein subunit rings. In fact, the RNA molecule sort of fits in a helical groove formed by depressions on the successive protein subunits (Fig. 4.6). This can be beautifully demonstrated in these particles by removing a limited portion of the protein coat thus exposing the central RNA core. Other important helical viruses show similar alignment, like sugar beet yellow, virus tobacco rattle virus and lucerne (Alfalfa) mosaic virus. Anisometric particles of some important animal viruses like the myxoviruses (influenza, parainfluenza etc.) are different in being enclosed within an envelop. However, the basic architecture of these particles is helical (Compan and Choppin 1971).

The tail of the tailed bacteriophages can also be regarded as

anisometric because of its helical configuration. Brenner and his co-workers (Brenner *et al.* 1962) demonstrated that the tail of bacteriophage of T even series has a multiple architecture in which a central hollow tube is surrounded by a protein sheath. With the aid of

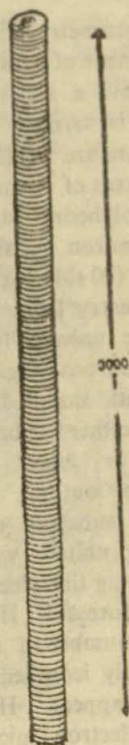


FIG. 4.5. Tobacco Mosaic Virus. This much enlarged diagram of the helical virus is rod shaped having a central core surrounded by a helical protein sheath. Note the arrangement of the spirals forming the sheath. There are 129 complete spirals in one sheath.

negative staining techniques it was revealed by them that the sheath showed 25 striations formed by parallel row of protein subunits. It has been estimated that about 200 *cog* like subunits make up these twenty five striations. (Fig. 4.7).

ARCHITECTURE OF ISOMETRIC PARTICLES

The cubic symmetry exhibited by many viruses requires that there be specific number of axes of symmetry about which the particles can be rotated to give a series of identical appearances. One type of cubic symmetry is termed 5:3:2 because there are 5-fold, 3-fold and 2-fold axes of symmetry in it. Two polyhedral structures, the dodecahedron (12 sides) and icosahedron (20 sides) exhibit this type of symmetry. Isometric virus particles are universally found to be icosahedrons (Fig. 4.3).

Viruses with icosahedral symmetry are either cuboidal or polyhedral or even roughly spherical in outline. This is because the subunits may be only loosely united with one another making the virus particles fairly deformable. However, greatest the number of subunits more genuinely icosahedral does a particle appear. However, under the electron microscope an icosahedron appears to be hexagonal. The nucleic acid of these viruses is surrounded by subunits forming the protein coat and generally follows the symmetry of the protein coat.

Detailed investigations into the icosahedral capsids reveal regularly spaced knob or ring like subunits or capsomeres separated by interstices. These subunit structures are also referred to as the major morphological units. The number of subunits forming the protein coats as icosahedrons in cuboidal viruses vary from 12 to 812 or even

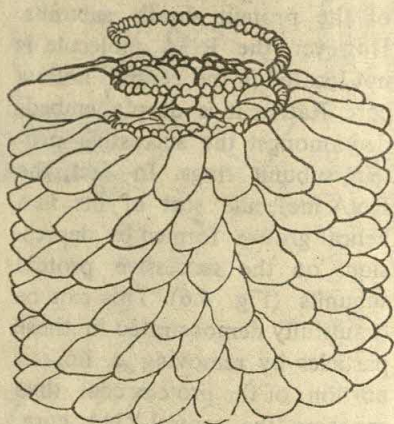


FIG. 4.6. Arrangement of nucleic acid and capsid in a helical virus (tobacco mosaic virus). Not the helical arrangement of the individual units of the capsid. The groove formed by them contains the nucleic acid. A Portion of the nucleic acid core is seen protruding out. The protein units forming the capsid remain as monomers and are not grouped into capsomeres. Rather, they are bound to each other forming a tape like uniform structure. (Modified after A. Klung and D.L.D. Kasper).

more. Each subunit in its turn, has been shown to be multimeric composed of a specific number of yet smaller basic units. Theoretical consideration and experimental data suggest the presence of two types of major morphological units; pentagonal *pentamers*, made up of five basic units or monomers and hexagonal *hexamers*, made up of six monomers. In either types the monomers form polygonal rings with central holes of various dimensions, upto 4 nm. The capsid is constructed by the assembly of capsomers according to specific geometrical pattern, wherein the pentamers form the corners of the icosahedron and the hexamers occupy the spaces in between (Casper and Klug 1962). Table 4.7 summarises the physical dimensions and Fig. 4.10 depicts graphically the assembly of capsomers of an icosahedral capsid.

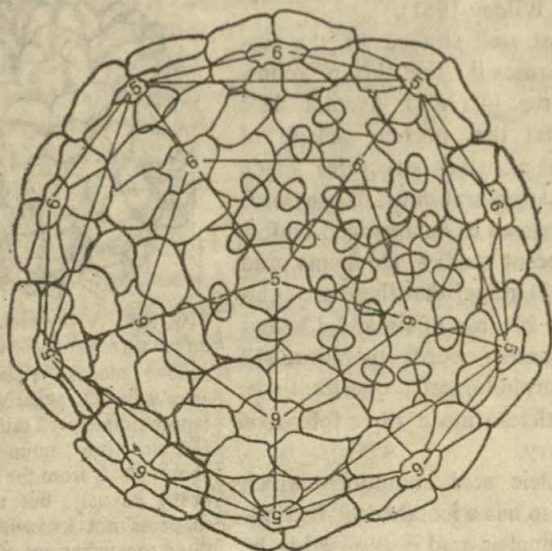


FIG. 4.7. Architecture of an icosahedral capsid. Note specifically the arrangement of the capsomeres. Each capsomere is made up of specific number of monomer as indicated in the centre of each of them. Usually, these are either Pentamers (made up of five monomers) or Hexamers (made up of six monomers). Both may be present in the same capsid.

Many plant viruses, like turnip yellow mosaic, tomato bushy stunt, tobacco necrosis and tobacco ringspot, which appear circular in outline under the electron microscope, have been shown to have a hexagonal contour. This indicated a icosahedral symmetry of these virus particles. X-ray crystallographic studies coupled with electron

microscopy have also confirmed the icosahedral nature of such viruses as turnip-yellow mosaic, tomato bushy-stunt and southern bean mosaic virus (Klug & Finch 1960).

Utilizing the technique of metal shadowing on freeze dried virus particles of *Tipula* iridescent virus, Williams and Smith (1958) demonstrated them to be icosahedrons when viewed in the electron microscope.

Animal viruses like that of the encephalomyocarditis group (EMC) have been shown to be icosahedral with probably two distinct protein groups (Housen and Shaffer 1964). Reoviruses have also been shown to be with icosahedral symmetry. Several other animal viruses like Polyoma, simian virus 40, herpes and adeno have been shown to be isometric with icosahedral particles (Horne & Wildey 1961).

The most well studied of the isometric viruses is the turnip yellow mosaic virus (TYMV). Studies have shown that the protein sheath of TYMV has a surface structures made up of 180 structural units. These were earlier supposed to be arranged uniformly but recent studies (Mellema and Amos 1972) reveal that these are clustered into 20 rings of six and 12 rings of five. In other words there are 32 large morphological organisations. These particles show a three fold axis of symmetry.

The nucleic acid component which is RNA also has a icosahedral distribution. The nucleic acid is supposed to be aligned along and embedded within the protein shell or its 32 gross morphological units (Fig. 4.8). X-ray diffraction studies confirm such embedded arrangement.

Cowpea chlorotic mottle virus (CCMV) shows an ultra-structure similar to that of TYMV except that it is much smaller.

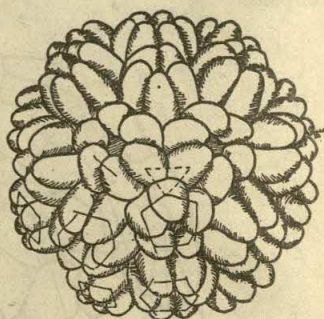


FIG. 4.8. A drawing of the outer surface of the turnip yellow mosaic virus particle is revealed by negative staining (approximately $\times 2$ million). The 180 structure units protrude about 20 Å from the main body of the particle, but their exact shape is not known. They are tilted somewhat out of the radial direction towards the directions of the three- and five-fold axes of the particle (although the clustering is not obvious in and-on views in negative stain images, because the centres of contrast of the units lie below the outermost surface). The RNA is associated closely with the hexamers and pentamers at an inner radius. (D.L.D. Caspar in J.T. Finch and A. Klug, 1966)

ISOMETRIC VIRUSES WITH MULTICOMPONENT STRUCTURE

Some recent reports have indicated that a virus particle may not be entirely homogeneous structurally but may sometimes be multicomponent with a divided genome.

As early as in 1949 Markham and Smith reported that turnip yellow mosaic virus (TYMV) could be separated out by ultra-centrifugation into a *top* and a *bottom* component; the former lacked nucleic acid and was not infectious whereas the bottom component had nucleic acid and was infectious.

In a recent study Geelen *et al.* (1972) have shown that cowpea mosaic virus (CPMV) is characterized by two types of heterogeneity. On the structural plane they are found to be constituted of three types of particles all icosahedral and of about the same size. However, they have different RNA contents and because of this have different sedimentation constants of 58s, 95s and 118s respectively. These are referred to as the top (*T*), the middle (*M*) and the bottom (*B*) components. The middle and the bottom components are nucleoproteins consisting 25 and 34 per cent of RNA respectively. Both these components are required for successful infection. Thus, there is some heterogeneity on functional plane as well.

Most viruses of CCPM group behave in the same fashion. Other viruses like bromegrass mosaic and raspberry ringspot mosaic viruses have been shown to be multicomponent (See Van Kamen 1972, Mathews 1970).

THE ENVELOP

In certain animal viruses and plant viruses an envelop is known to surround the virus particles. This envelop is a membrane about 10 to 15 nm thick. Chemically the envelop is made up of proteins, lipids and carbohydrates, which appear to be specifically aligned as lipoproteins and glycoproteins. The presence of lipids makes the envelop loose and flexible which indirectly leads to the presence of particles of different sizes and shapes. The envelop is composed of both host and viral components, the protein tending to be virus specific. The lipid and perhaps also the carbohydrate component may be characteristic of the host membrane (nuclear, vesicular or cytoplasmic).

NOMENCLATURE OF THE VIRUS PARTICLES AND ITS PARTS

In the early days of virology, each particle appeared as a structureless mass, even on electron microscopy. Therefore, no necessity for descriptive names for a particle or its parts was felt. However, as more and more information regarding virus morphology and architecture was gathered, a set of nomenclature became increasingly necessary. Lwoff *et al.* (1959) suggested such a nomenclature for the first time. This was further expanded by Casper *et al.* (1962) and promulgated at the Cold Spring harbour symposium of 1962.

A virus particle capable of infecting a specific host organism is termed a *Virion*. Capacity to infect has come to be regarded as a genetically controlled characteristics in viruses. There are instances where the infectiveness of viruses is known to have been lost though no apparent symptom of the loss is discernible. Virus particles with or without the capacity to infect but otherwise complete in other respects is referred to as *Nucleocapsid*. The two major components of a virus particle, namely, the nucleic acid and the protein sheath, are known as the Core or the *Nucleoid* and *Capsid* respectively. The capsid is formed by a number of structural subunits called the *Capsomeres*. These are made up of polypeptide chains, numbering two to four coiled in a specific manner. The number of capsomeres and their proper alignment are specific and are characteristics of individual capsids. Some animal and plants viruses have a lipoprotein membrane surrounding them. The membrane structure is called the Envelop. These are often projection on these envelop known as *Spikes*. These spikes may be specifically arranged into distinct units. Such membranes or the units forming them are called *Peplomers*. These descriptive terminologies are useful to considerable extent in the proper elaboration of individual viruses. Additional information obtained are no doubt going to amend and altered them in the days to come.

MORPHOLOGICAL CATEGORIES IN VIRUSES

We have seen in the previous paragraphs that the viruses exhibit but a few specific morphological characteristics. Most of them can be grouped in one category or the other in accordance with these characteristics. These categories, however, cannot be considered to be taxonomic groupings since often widely different viruses may possess similar or even identical morphological attributes.

One of the convenient ways of categorising viruses would be by

taking into consideration their symmetry and the presence or absence of envelop around them. Some viruses, notably the pox viruses and a few of the arboviruses would, however, be necessarily left out of such a scheme. This would be so because of the unusual symmetry possessed by virus particles belonging to these groups.

Accordingly the viruses could be categorised into the following principal morphological groups (See Fig. 4.1).

- (1) Naked elongated (helical) viruses.
- (2) Naked spheroidal (icosahedral) viruses.
- (3) Enveloped elongated (helical) viruses.
- (4) Enveloped spheroidal (icosahedral) viruses.
- (5) Tailed (binal) viruses.
- (6) Uncertain types.

Naked Elongated Viruses

Elongated, non-enveloped particles are observed mostly in plant and bacterial viruses. Common examples are, tobacco mosaic virus (TMV), tobacco rattle viruses (TRV), Potato virus X and Coliphage fd (See Table 4.3). These particles are more long than broad, often by as much as 18 folds e.g., TMV which is 300 nm long and 18 nm in diameter. The particles may be rodlike and rigid (TMV) or filamentous and flexuous as in coliphage fd.

Most studied of the naked elongated viruses is the tobacco mosaic virus. It is now established that it is a nucleo-protein particle of 40×10^6 daltons with one central single stranded RNA molecule surrounded by a protein sheath. The protein is a single species but is made up of about 2300 identical units each with a molecular weight of 18000 (Harris and Knight 1955). The nucleic acid has a molecular weight of about 2×10^6 and is about 3300 nm long (about 10 times the length of the particle) and is situated centrally.

X-ray diffraction pattern analyses (Franklin *et al.* 1957; 1959; Casper 1962) revealed that the protein subunits of the virus particle are arranged in a helical array about the long axis of the rod. There are about 130 turns per rod comprising of about 2300 subunits. It was also revealed that there was a central hole of about 4 nm diameter making the rod, in effect, a tube. The nucleic acid is not at the Centre of the tube but rather is interwoven in the protein subunits at a radius of about 4 nm (Plate VI).

Enveloped Elongated (Helical) Viruses

A large number of animal and plant viruses would fall under this category. All of them share the feature of maturing at cell membranes

through which they bud. Thus they acquire an envelop around them. The envelop is composed of both viral and host cell components; the protein is usually virus specific while the lipids and carbohydrates are the characteristic of the host membrane. Quite often the membranes have spikes on them. An interesting feature about these viruses is that while the nucleocapsid particles exhibit a helical configuration, the enveloped virus may be spheroidal *e.g.* some myxoviruses like sendai virus.

In these viruses the helical nucleo-capsid exists in a folded or coiled state within the envelop. Sometimes, however, they may show morphological plasticity leading to a sort of pleomorphism. There may even be fragmented nucleocapsid as in sendai virus and in oncornaviruses.

Some plant viruses like the vesicular stomatitis virus and potato yellow dwarf virus are other examples of helical enveloped viruses.

Naked Spheroidal (Icosahedral) Viruses

Many plants, animals and microbial viruses have a naked spheroidal shape. As for example, turnip yellow mosaic virus (TYMV), brome mosaic virus (BMV), poliovirus, adenovirus-5, ϕ X174 and coliphage Q β (see Tables 4.4 and 4.5).

Adenoviruses are one of the larger spheroidal viruses which have been extensively investigated into. These are about 80 nm in diameter. The outer protein coat has 252 morphological units each consisting of either five or six subunits. The cluster formed by five subunits are termed *pentons* or *pentamers* and those with six subunits are known as *hexons* or *hexamers*. There are 32 hexons which all are roughly polygonal discs of 8 nm diameter with a central hole which 2.5 nm across. Each hexon is surrounded by six more hexons thus building up most of the icosahedral particle. There are 12 pentons situated at the 12 vertices of the icosahedral and each is bounded by 5 hexamers (See Fig. 4.7 and Fig. 4.8). Detailed analyses revealed that each penton serves as base structure to which fibers called penton fibers are attached. Each penton fiber is about 2×20 nm in diameter and terminates in a spherical knob about 4 nm in diameter. These fibers have important serological properties (Horne *et al.* 1959; Horne 1973).

Another well studied naked spheroidal virus is the turnip yellow mosaic virus (TMYV) which has been described earlier in this chapter. A special feature revealed by X-ray crystallographic studies on these particles concerns the alignment of nucleic acid with the protein (see Fig. 4.8). It has been suggested that there is a regular interlacing of the nucleic acid with the protein subunits deep enough below the

surface.

Another interesting example of naked spheroidal viruses are the reoviruses. These viruses have been shown to be consisting of a segmented double stranded RNA surrounded by two successive layers of protein shells, an inner and an outer. It is not clear whether the outer shell is part of the nucleocapsid particle or is an independent covering.

Not much is known regarding the alignment of nucleic acid strands with the protein coat in these viruses excepting the turnip yellow mosaic virus. However it is reasonable to assume a non-covalent association between the two (Knight 1974).

Enveloped Spheroidal (Icosahedral) Viruses

This group also includes several plant and animal viruses and they share with the helical enveloped virus their mode of maturation and budding.

Herpes viruses are the most well known examples of enveloped icosahedral viruses (see Table 4.2). Some myxo virus like the influenza virus and oncornaviruses like Rous sarcoma virus are the other examples. Some arbo viruses (Group A) are also spheroidal with surrounding envelopes.

The exact nature of the protein sheath in these viruses and their relationships with the nucleic acid component are not clear as yet.

The bacteriophage *Pseudomonas* 2(PM_2) is icosahedral with an envelop covering it. This is known to have a double stranded circular DNA molecule.

Tailed (Binal) Viruses

While some bacterial viruses are spheroidal (ϕ X174) and some elongated (coliphage fd) a large number of them are combinations in which head and tail structures are clearly discernible. The head is usually spheroidal (icosahedral) and the tail elongated (helical) in symmetry.

The most well known example of tailed viruses are the bacteriophage of the *T* series. The head in these particles houses the nucleic acid and varies considerably in shape and size. The tail serves as an attachment organ in the initial steps of infection. The tail has tubular structure surrounded by a sheath like covering. The tube penetrates into the host cell during infection and being hollow allows the viral DNA to pass into the host cell. The tail sheath may (*T* even) or may not (*T* odd) be retractile. These particles are extremely complex and have an array of such accessory structures as collars, base plates, spikes, tail-fibers and so on (Table 4.5 and Fig 4.4).

Tailed viruses are also exemplified by infectious agents of blue-green algae (cyanophages). Their structure appears to be similar to bacteriophages and exhibit a similar range of variation.

Uncertain Types

A few viruses possess different and more complex morphology than encountered in the members of any of the group of viruses mentioned earlier.

Poxviruses are the largest of most complex of such viruses (Fig. 4.9). Their virions are usually described as brick or loaf shaped having a size of 250 nm by 300 nm. These viruses, irrespective of their respective sources, have a tubular, highly convoluted lipoprotein outer membrane. This surrounds an internal protein-nucleoprotein core also called the nucleoid. Between the central nucleoid and the outer membrane are two lateral proteinaceous bodies on either side. The two lateral bodies cover the nucleoid partially in the central region alone. The nucleoid itself appears to be surrounded by a membrane (Easterbrook 1966).

These viruses are reported to possess several enzymes including DNA destroying nucleases. These enzymes are present with the nucleoid but are supposed to remain inactivated by the presence of the lateral bodies (Dales 1977).

Some vaccinia viruses *e.g.* 'orf' (contagious pustular dermatitis) virus have a crossed helical pattern on their surface (Waterson 1968).

Encapsulated or Occluded Viruses

A few insect viruses appear in their natural form in characteristic inclusion bodies. The nuclear polyhedroses and the cytoplasmic polyhedroses viruses are two well known examples of such viruses.

These inclusion bodies are one generally crystalline protein packages which contain one or two or more virus particles. Some of these packages are called polyhedral bodies and are found characteristically in either the nucleus or the cytoplasm of host cells. Therefore, the names of the diseases caused by them *i.e.* nuclear polyhedrosis and cytoplasmic polyhedrosis.

The occluded virions of these viruses may be either spheroidal (cytoplasmic polyhedrosis) or elongated (nuclear polyhedrosis). The number of particles in the crystalline matrices are many in these viruses. However, in another similar virus, the granuloses virus, the number of virus particle per inclusion body or capsule may be only one.

Thin sections of polyhedral viruses and capsules reveal that two concentric membranes surround each particle. The exact nature and significance of these structures are not very clear.

Chapter Five

PHYSIOLOGY OF VIRUSES

Viruses are nucleoprotein particles which are obligately and totally parasitic upon their hosts. Though they cannot survive alone yet, they are not by any means to be considered passive cellular inclusions passing time at the expense of the host cells. They are, on the contrary, extremely active within the host cell, weaving out a complex pattern of a series of physiological activities culminating in the production of a large number of particles of the same genetic types. In fact they achieve all this not with the support of or at the bidding of the host cell but, first, by overcoming the resistance offered by the latter and secondly, by converting the latter into a subservient entity.

Once within the host cell, viruses are almost always the masters of the situation unless they choose to be otherwise and remain in a sort of peaceful coexistence with the host cell chromosome. The complex and specific array of activities that the viruses perform inside a cell are truly staggering. These activities are yet to be fully elucidated. Virologists are paying increasing attention to this in recent years. For it is obvious that a proper understanding of the viral situation is, most probably, the only key to appreciating life at the molecular level.

Functions and behavioural characteristics of viruses are dependent essentially upon the chemical constituents comprising the virus particles. The individual constituents namely, the proteins, the nucleic acid, the lipids and the carbohydrates all have a role to play, either separately or in combination. However, the nucleic acids and the proteins, being the major constituents universally occurring in viruses, have major roles to play in the physiology of viruses. The other components have only subsidiary or accessory roles, which nevertheless are not unimportant. The interactions with the host cell also act as modifying factors.

FUNCTIONS OF NUCLEIC ACIDS OF VIRUSES

The widely accepted view that the nucleic acids, particularly DNA (deoxyribo nucleic acid) is the genetic material in all living organisms is equally true of viral nucleic acids. Researches have revealed that

both viral DNA and RNA (ribonucleic acid) act as the genetic material in viruses containing the respective nucleic acid types.

Working with a plant virus, the turnip yellow mosaic virus (TYMV), Markham and Smith (1949) demonstrated that on centrifugation at high speed, a supposedly homogeneous preparation of this virus resolved into two components. The major component was found to have a sedimentation coefficient of 106 *S* and comprised about seventy five per cent of the material by weight. The minor component had a sedimentation coefficient of 49 *S* and formed about twenty five per cent of the material. The most important observation, however, was that the major heavier component had about thirty seven per cent RNA and was infectious. The minor component had no RNA at all and was non-infectious.

Markham and Smith concluded from this observation that "the role of the protein constituent of plant viruses is undoubtedly very important but there is some evidence that nucleic acid is in fact the substance directly controlling viral multiplication" (Markham 1953). However, it was pointed out by them that since the lower component had both nucleic acid and protein, it could be that both the components were necessary for making the particle multiply and become infectious. Though of great significance, the work of Markham and Smith did not conclusively prove that nucleic acid was the genetic material in viruses.

The conclusive direct evidence proving that the nucleic acids indeed were the genetic material in viruses came with the classical work of Prof. A.D. Hershey and Margaret Chase (1952) on the process of infection of the bacterium *Escherichia coli* by coliphage *T*₂. Using phage whose proteins were labelled with *S*³⁵ and whose DNA contained *P*³², they showed that more than 80 per cent of the radioactive sulphur (and hence protein) remained on the outside of the infected cells whereas only 20 per cent or even less of the phosphorus (and hence nucleic acid) remained outside (Fig. 5.1).

They further showed that even if the bulk of the surface radioactivity (and hence proteins) was removed mechanically, the cells went ahead and reproduced *T*₂ phages which showed radioactivity in their nucleic acids (from powerful radioactive phosphorus incorporated into DNA). Performing the experiment with separately labelled coliphages, they came to similar results. Their conclusion was that DNA probably exercised the genetic function of the phage and that the protein acted as a protective sheath for the DNA without having any genetic significance.

The evidence that the nucleic acid alone, without the benefit of the protein coat, is capable of successful infection and multiplication was

provided by Franenkel-Conrat in 1956. Working with tobacco mosaic virus (TMV) he reported that RNA preparation obtained from TMV

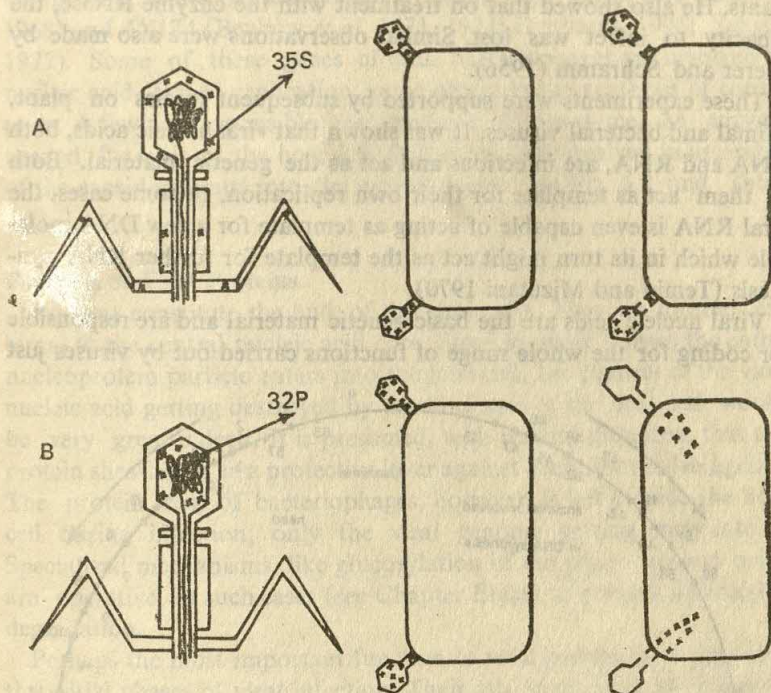


FIG. 5.1. The Hershey-Chase experiment. This was designed to show that the nucleic acid and the protein are the infectious and the non-infectious components of a virion respectively. Diagram (A) depicts a situation where phage particles were originally cultured in a medium containing amino acids having radioactive sulphur (S^{35}). Therefore, the capsids of these phages were labelled. The labelled phages were then made to infect fresh host bacteria for about 10 minutes. The bacterial culture was then blended vigorously in a homogenizer. The 'ghost' protein coats of the adsorbed virions dropped off and were separated out. These 'ghosts' were found to contain radioactivity. The progeny of these phages did not contain any radioactivity whatsoever. This showed that proteins were not involved in infection.

In another set-up, phage particles were originally grown in media containing radioactive phosphate (P^{32}). Since phosphate constitutes a part of the phage nucleic acid, the progeny of these phages all had radioactive nucleic acid. The progeny phages were then made to infect fresh host bacterial cells. These were incubated for ten minutes. Thereafter, these were also blended as earlier. The 'ghost' proteins were separated out. The host bacterial cells contained all the radioactivity. The phage progeny liberated after lysis of the bacterial cell also contained radioactivity. This showed that the nucleic acid was the infectious component of a virion.

was infectious in itself and could cause the disease in healthy tobacco plants. He also showed that on treatment with the enzyme RNase, the capacity to infect was lost. Similar observations were also made by Gierer and Schramm (1956).

These experiments were supported by subsequent studies on plant, animal and bacterial viruses. It was shown that viral nucleic acids, both DNA and RNA, are infectious and act as the genetic material. Both of them act as template for their own replication. In some cases, the viral RNA is even capable of acting as template for a new DNA molecule which in its turn might act as the template for further RNA synthesis (Temin and Mizutani 1970).

Viral nucleic acids are the basic genetic material and are responsible for coding for the whole range of functions carried out by viruses just

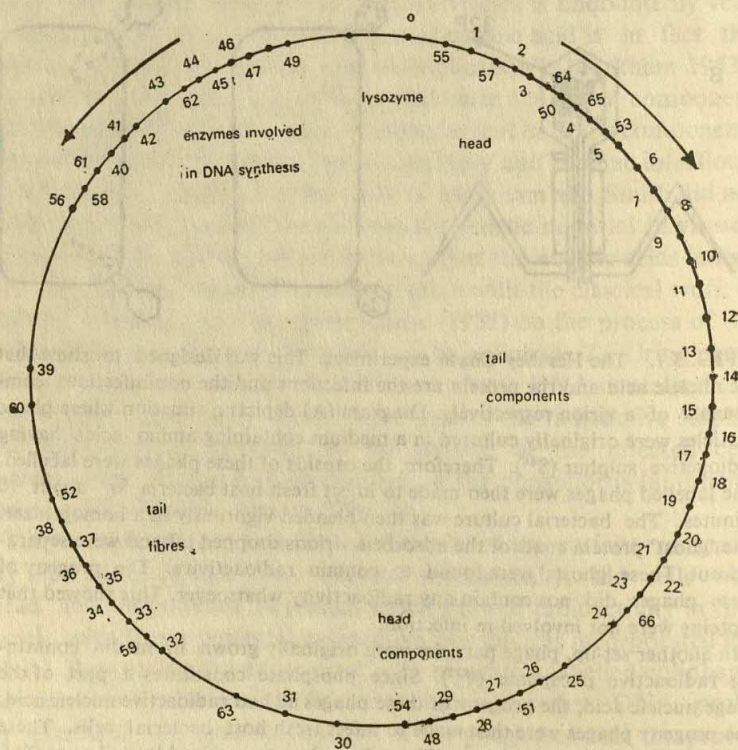


FIG. 5.2. A diagrammatic representation of T₁ Genome (after King, 1968).

as is the case in undoubtedly living organisms. The different regions of the viral nucleic acid sequence are responsible for initiating and

completing specific functions. These regions or the genes have well mapped out in the bacteriophages T_4 (King 1968, Edgar and Lielausis 1968) and $\phi X174$ (Benbow *et al.* 1971, 1974; Denhardt 1975; Sangar 1977). Some of these genes of these bacteriophages are involved in nucleic acid (DNA) replication; some others in the synthesis of capsid units. A few are responsible for synthesis of lysozyme, an enzyme needed for lysis of the host cell. Still others are involved in assembly of the capsid subunits into the protein coat (see Fig. 5.2 and Table 3.7).

Function of Viral Proteins

Proteins constitute the bulk of the viral mass. They are located external to the central nucleic acid core. Since in many viruses the entire nucleoprotein particle enters into the host cell, the chances of the viral nucleic acid getting destroyed by the nucleases of the host cell would be very great indeed. It is presumed, with reason, therefore, that the protein sheath acts as a protective layer against such destructive agents. The protein coat of bacteriophages, however, is left outside the host cell during infection, only the viral genome getting entry into it. Specialized mechanisms, like glucosylation of the phage nucleic acid, are operative in such cases (see Chapter Eight) to prevent destructive degradation.

Perhaps the most important function of viral protein is to negotiate the initial phases of viral infection. Their job, in effect, is to recognise the specific host cell and align the entire nucleocapsid particle in a manner most conducive to the process of infection. Proteins thus play an important role in establishing the host specificity of a given virus.

The basis for such specificity of alignment is the attachment of a virus particle to receptor site on its host cell. This is particularly true for animal and bacterial viruses. A specific viral protein is involved in this attachment. For instance in the case of T series of coliphages, the tail fibers, which are proteinaceous, serve as specific attachment organs. Phages whose tail fiber proteins have affinity for receptor sites on the bacterial cell wall can attach and initiate infection. In the cases of tailless bacteriophages, like coliphages f_2 and f_r , the coat proteins are specific and themselves play the role required for attachment of the virus particle to the host cell and initiatable infection.

The same phenomenon appears to be true of animal viruses also. An example of such specificity is provided by poliovirus. As intact virion, poliovirus has a restricted host range, namely the primate cells. This restriction is known to be dependent on the specific affinity between poliovirus coat protein and the receptor material on the host

primate cell surface (Holland 1964). However, when the poliovirus RNA alone is used as the infectious agent, the host range of the virus is vastly expanded to include other vertebrates like mice, rabbit and chicks.

Viruses have antigenic properties and are capable of inducing production of antibodies in animals. This is generally achieved on introduction of the virus particle into the animal. These antibodies can react with the respective viruses in a variety of immunological or serological ways. This property is essentially dependent upon the protein coat; specifically, on the physico-chemical nature of its subunits. Glycoproteins and lipoproteins are also capable of eliciting similar response from the host cells.

Apart from the capsid proteins, certain complex viruses, like the pox viruses and the bacteriophages, contain non-coat protein constituents as well. These proteins are mostly enzymic. Two well-known examples are the phage lysozyme and myxoviral neuraminidase. These enzymes break down the cell wall or membrane constituents of the host cells (Kozloff 1968; Webster 1970). Some other non-coat protein enzymes are RNA transcriptase of reovirus (Shatkin and Sipe 1968), RNA polymerase of Newcastle disease virus (Kingsbury 1972) and 'reverse' transcriptase of Rous sarcoma virus Temin and Mizutani 1970). Another group of enzymes, the protein kinases have been reported from a number of animal viruses including RNA tumour, influenza, vaccinia and herpes viruses (Rosemond and Moss 1973). These enzymes are supposed to attach phosphorous group to the proteins.

Role of Lipids and Carbohydrates

Lipids are associated with the membrane of the enveloped viruses. Selective degradation of lipids by diethyl-ether leads to substantial degradation of the virus particles (Waterson 1968). Obviously, therefore, the lipids are one of the essential elements in maintaining the structural integrity of enveloped viruses especially their membranes. Distortion of the structural features leads to loss of infectivity as well. This is probably due to the attendant failure of the virus particles to attach to the host surface and penetrate subsequently. This is particularly the case in animal viruses; the role of lipids in plant viruses is not very clear.

Viral carbohydrates are present as glycoproteins and glycolipids in enveloped viruses. They constitute an important component of the spikes of myxoviruses. In these viruses the carbohydrates present are of two types; one constitutes the haemagglutinin of these viruses and

the other is associated with the neuraminidase enzyme systems (Compans and Choppin 1971). If the carbohydrate residue is removed from them, there is a distinct loss in the haemagglutination capacity (Bikel and Knight 1972). Since these carbohydrates form an integral part of the viral envelop, they are also suspected to play a role in specific attachment of the virus to the host cell and probably also in their release from the host cell (Knight 1974).

Glycoproteins, as mentioned earlier, have a role in the immunological reactions of animal viruses. The immunological behaviour of purified influenzal haemagglutinin and the glycoprotein isolated from vesicular stomatitis viruses have been found to be similar to that of influenzal virus and vesicular stomatitis virus respectively.

Simple sugar like glucose or gentiobiose are often found associated with *T* even coliphage DNA. It has been found that such glucosylated nucleic acid is resistant to action by host cell nucleases. This resistance mechanism seems to be particularly necessary for *T* even phages infecting some *E. coli* strains; other non-glucosylated phages do not seem to be much affected and multiply and produce progeny in these bacteria (see Chapter Six).

Role of Other Constituents

Polyamines and metallic cations are known to be associated with viruses in small amounts. The polyamines putrescine, spermine and spermidine have been found in the nucleic acid of bacteriophages T_2 and T_4 and are supposed to play an important role in the alignment of nucleic acid within the protein coat by helping it to fold in a specific and predetermined fashion (Cohen and Dixon 1971). However, because of their absence in many other viruses (including other bacteriophages), such a role remains doubtful (Knight 1974). The role of metallic cations is also not very clear but they might be affecting the conformational features of viral nucleic acids and proteins.

REPLICATION IN VIRUSES

In the preceding paragraphs we have attempted to focus upon the roles the individual constituents of viruses play. It need not be emphasised, however, that their respective functions are neither arbitrary nor do they occur haphazardly. Rather there is a coordinated pattern of metabolic functioning within and in association with the host cell which culminates into specific physiological phenomena like viral reconstitution and multiplication of the same genetic type of virus.

Reconstitution of Viruses

The phenomenon of reconstitution may be characterized as the bringing together of protein and nucleic acid components of a virus in such a manner that they recombine specifically to yield homogeneous virus particles. Ideally reconstituted particles depict most of the properties, including that of infectivity, of the parental virion. This process is sometimes referred to as *self-assembly* indicating that it takes place, autonomously as it were, outside the host cell system. It should be remembered that this process is not a naturally occurring phenomenon although it is primarily dependent upon the basic characteristics of the reuniting protein and nucleic acid moieties.

As early as in 1947 Schramm (1947 *a, b*) reported that the dissociated proteinaceous particles of tobacco mosaic virus (TMV) could reunite under appropriate conditions. A few years later, Takahashi and Ishi reported that proteins isolated from TMV infected plants could be aggregated into rod like particles which looked very much like TMV particles as seen under the electron microscope. These reconstituted rods, however, lacked the power of infection (Takahash and Ishi 1952).

The first successful attempt at reuniting purified protein and nucleic acid components of some strains of TMV was made by Fraenkel-Conrat and his colleagues a few years later (Fraenkel-Conrat and Williams 1955). In a series of ingenious experiments, they first isolated purified TMV preparations from TMV infected tobacco plants. They then obtained purified RNA and protein from these samples utilising suitable extraction procedures.

They found that the protein thus prepared was composed of three subunits which tended to aggregate under neutral pH condition. They called it protein *A*, and found it to have a molecular weight of around 53,000. Protein *A*, however, was found to be non-infectious.

The RNA isolated was found to be of much higher molecular weight (2×10^6 dalton) and was slightly infectious. When the RNA and the protein *A* were combined at pH 7.3 and incubated for 1 hr at 30°C, reconstituted rod like TMV particles were formed. These particles were shown to be highly infectious with molecular weight of 40×10^6 daltons. Several other properties of reconstituted particles like sensitivity to heat, altered pH conditions were found to be of similar nature to that of TMV particles (Fig. 5.3).

Since this initial demonstration a large number of investigations have been reported on various aspects of viral reconstitution. The findings accumulated may be summarised as below:

It has been shown that mixed reconstitution can be achieved by

using the nucleic acid of one strain and the protein of a related strain belonging to the same species. However, mixed reconstitution is not fully achieved with the nucleic acid and the protein components of different species or even divergent strains of the same species of virus. In fact, in certain cases even slight, but obviously important, differences in the amino acid composition can be responsible for non-reconstitution.

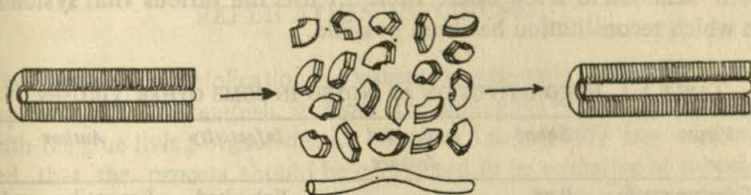


FIG. 5.3. Reconstruction of viruses. Tobacco mosaic particles were disintegrated using mild methods. This resulted in the disintegration of the capsids. When such broken pieces were mixed with the nucleic acid core again, complete particles were soon formed. In this manner reconstitution of viruses might take place. Under certain conditions, protein particles of one type could be aligned with the nucleic acid of another type. However, the infectivity of such hybrid particles is determined by the nucleic acid alone.

Water too plays an important role in the aggregation of protein subunits into TMV like rods. Polymerization of TMV protein *A* has been shown to be accompanied by release of water molecules. It has been suggested that before aggregation, the protein subunits are in an aquatic environment. But during aggregation they gradually change to an organic environment of the other aggregating subunits. The protein aggregate is held chiefly by hydrophobic interactions between the subunits, a process which is facilitated by the release of water molecules. The process of aggregation of subunits is essentially endothermic, the energy probably being provided by the concomitant dissociation of water molecules from protein subunits.

Mechanism

The question as to how exactly the protein subunits and the nucleic acid aggregate has also been investigated into. In a study of assembly of tobacco mosaic virus *in vitro*, Richards and Williams (1972) reported that under proper ionic and pH conditions there is a spontaneous polymerization of thirty four TMV protein subunits into what appeared to be discs. The further observed that the formation of such discs was essential for rod initiation. Subsequent addition of other units then results in rod elongation. The units added later may be larger or smaller than the initiation unit. Such an *in vitro* aggregation of subunits

into protein *A* is very similar to the process occurring during replication (Butler and King 1972). The addition of protein subunits appears to proceed from the 5'-end of the viral RNA to the 3'-end.

Partial to total reconstitution of many other plant, animal and bacterial viruses have been reported. All these viruses reconstitute under conditions which are akin, if not exactly identical, to those needed for reconstituting TMV particles. Heterologous reconstitution has also been achieved in a few cases. Table 5.1 lists the various viral systems in which reconstitution has been possible.

TABLE 5.1. RECONSTITUTION AS NOTED IN SOME OTHER VIRUSES

| <i>Virus</i> | <i>Shape</i> | <i>Special feature</i> | <i>Infectivity</i> | <i>Author</i> |
|---|--------------|---|--------------------------|------------------------------|
| Tobacco rattle virus | Rod | — | Exhibited | Senancik and Reynolds (1969) |
| Brome mosaic virus | Spheroidal | Can. associate with widely different nucleic acids types. | Exhibited | Hiebert <i>et al.</i> (1968) |
| Coliphage fd | Spheroidal | Maturation protein essential | Exhibited | Hohn and Hohn (1970) |
| Poliovirus | Spheroid | — | Exhibited | Drzeniek and Billelo (1972) |
| Coliphage T ₄ | Binal | Specific enzyme required | Exhibited but not always | Edgar and Lielausis (1968) |
| Coliphage lambda | Spheroid | Specific protein (F) required | Exhibited but not always | Weigle (1966) |
| <i>Salmonella</i> phage P ₂₂ | Spheroid | — | Exhibited | Israel <i>et al.</i> (1967) |

As mentioned earlier, the phenomenon of reconstitution has been observed only *in vitro* i.e., outside the host cell. This phenomenon is, therefore, essentially a function of the isolated viral macromolecules, viz. the proteins and the nucleic acids. However, the process is not entirely a spontaneous physical phenomenon and is by no means a simple physical association between the protein subunits amongst themselves or between and the nucleic acid component. There is also a biological dimension i.e. the capacity of infection. If the physically reconstituted particles are not infectious, reconstitution as a special phenomenon cannot be said to have occurred. Researches have shown that for the reconstituted particles to be infectious, they must be assem-

bled properly out of the constituent units of the virus in a physical sense and also the process must be mediated by special agents like *F* protein or maturation protein to give them the biological property. Reconstitution, therefore, is a physical process merging into a biological phenomenon at a given point of time. Detailed analysis of this process have been of immense value in elucidating the nature of assembly of virus during replication.

REPLICATION IN VIRUSES

The process of multiplication in viruses is generally referred to as replication to distinguish it from the analogous process associated with the true-living organisms. Dr Salvador Luria (1967) has suggested that the process should be considered to be operative at subcellular level, more or less as a variation of the macromolecular synthetic process. Professor N.W. Pirie has called it "a mere exploitation of the metabolic faculties of the host cells and not a separate process." However, from whatever angle one might look at it, there is no escape from the reality of multiplication in numbers of specific particles. The fact that a living host cell is imperative for the process, merely makes it unique.

Our knowledge concerning this phenomenon has been mainly acquired during the last two decades and has principally been based upon the behaviour of bacterial viruses. Bacteriophage-bacteria systems have been adopted as a technically suitable model system from the very early days of virology. It proved quite effective in studying the complex process of viral multiplication and has since been used in the understanding of the process in a large variety of host-virion systems differing morphologically and genetically. Excellent comprehensive treatment of the phenomenon can be found in Cohen (1963), Levintov (1965), Stolph and Starr (1965), Stone (1969), Reichman and Clark (1968), Lodish (1969), and Waterson (1968).

Early Works

Earlier studies were mainly exploratory in nature. During the early forties, Max Delbruck and his colleagues had shown that growth of bacteriophages could be correlated with the lysis of the host bacterial cells (Ellis and Delbruck 1939). During the forties several studies were made by the same group of workers which clearly brought out that the phenomenon of specificity of infection of bacteriophages and their mutability (Delbruck and Bailey 1946).

Investigating the effect of mild detergents on purified plant viruses,

K.M. Smith and his associates had noted that on high speed centrifugation, detergent treated particles segregated into a less heavy and a more heavy component. The former was proteinaceous and non-infective and the latter was a nucleic acid and invariably infectious (Markhem and Smith 1949). Similar observations were also recorded by H. Fraenkel-Conrat during the fifties in relation of tobacco mosaic virus (TMV) particles.

In 1952 it had been shown by Alfred Hershey and Martha Chase that the protein part of the bacteriophages did not enter the host bacterium but the nucleic acid did and that the non-entry of the former did not in any way interfere with the process of multiplication. Though apparently unrelated, these studies went a long way in establishing the foundations of subsequent investigations.

MULTIPLICATION IN BACTERIOPHAGES

Multiplication of bacteriophages has been chiefly elaborated by Drs Max Delbruck and S. Luria and their associates in the United States and by Professor Andre Lwoff's school in France. These workers mainly concentrated on the *T*-phages of the bacterium *Escherichia coli*, particularly the *T*-even phages. Among the other coliphages employed for studying certain special aspects, the bacteriophages Lambda, ϕ X174 and f_2 are worth mentioning.

It has been suggested by Lwoff that bacteriophages exist in three clear cut states, namely, as *Extracellular Virions*, *Vegetative Phage* and *Prophage* (Lwoff 1962). The extracellular virions are the complete particles as existing prior to infection. Both the vegetative phage and prophage are intracellular, and as will be seen, are only nucleic acid. As vegetative phage the nucleic acid is capable of autonomous replication. As prophage it is inserted in the bacterial chromosome, or to be more precise, the bacterial DNA and remain in a latent state. Further, it is replicated with the bacterial DNA.

All bacteriophages exist as vegetative phages in the host cell for some time but not all become prophages. Those able to become prophages are called *temperate* and those unable to do so are known as *virulent*. Our information concerning the multiplication of bacteriophages is based mainly on the behaviour of virulent *T*-even phages infecting *E. coli*. The behaviour of temperate phages will be taken up later and in the meanwhile we shall consider the multiplication process of virulent phages, generally referred to as the lytic cycle.

THE LYTIC OR THE GROWTH CYCLE

The replicative process of virulent phages is called the lytic cycle because at the end of it the host bacterial cell is lysed or destroyed. This process can be divided into several distinct phases, namely, those of Adsorption, Penetration, the Eclipse, the Latent Period and Lysis, occurring in that order. Along with such destruction of the bacterial cell, there is a simultaneous release of virus particles. The phase initiating with the infection of the host cell by a phage and culminating in the release of newly synthesized viral particles has come to be known as the growth cycle and is depicted as what has come to be known as the *one step growth curve* (Fig. 5.4 on page 110). This type of growth curve was first introduced by Ellis and Delbruck (1939).

Adsorption

Attachment of the virion to the host bacterium is called *adsorption*. It occurs in two steps: collision between virions and the bacterial cells and subsequent establishment of specific association of virions with the cell surface. The first is dependent upon the concentration of the virions and the second upon the affinity that must exist between the virion and the cell surface.

This affinity is determined by the chemical nature of and charge distribution on the host cell wall. The latter property makes the cell wall antigenic and, therefore, only specific types of phages are able to infect a particular host cell and vice-versa. In certain coliphages these affinities exist not at random but only at certain regions on the wall called the *receptor* sites. Often these sites are present not on the cell wall surface on the outer side but deep within; sometimes there may be layers of receptors. The receptors are specific identifiable entities. For instance, receptors for T_5 coliphages have been isolated in the pure form and are known to be constituted of proteins and lipopolysaccharides.

The bacteriophages also have specific and specialized sites for adsorption. In the case of *T-even* coliphages these are the tail fibers. Isolated bacteriophage *T-tail* fibers are reported to get adsorbed on the bacterial surface whereas the fiberless phages fail to do so. Once adsorbed the phage particles become irreversibly attached to the cell surface. The mechanism of such irreversibility is not very clear (Plate IX).

Penetration

The next phase is that of penetration or injection of the phage

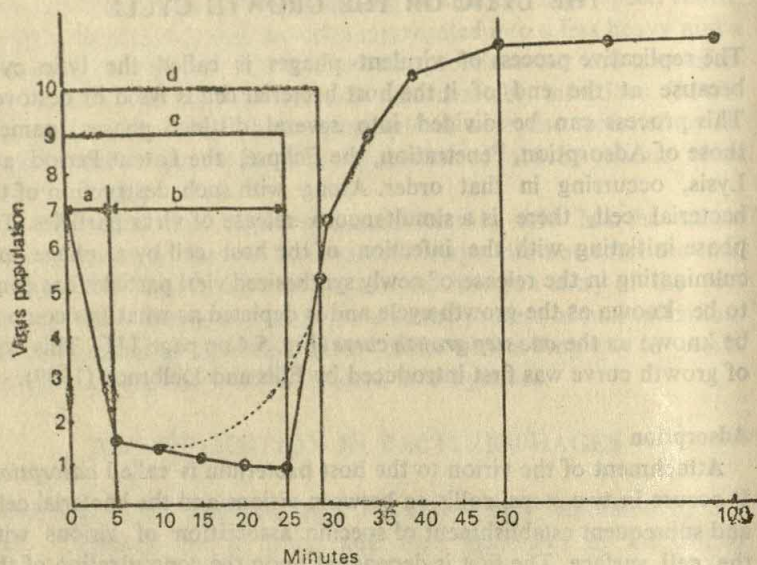


FIG. 5.4. Representative growth curve of bacteriophage *T*₂ in bacterial cultures. Young actively growing cells were inoculated with a suspension containing 10^6 to 10^7 particles per ml. Each of these particles was capable of infecting a host cell. Within a few minutes, most of the virus particles were absorbed on to and penetrated into the host. Infective virus particles virtually disappeared from the suspension fluid. Very little activity of infectious virus particles was visible (*a*). This period of non-activity continued for about 15 minutes and is called the eclipse (*b*). But, during this period, steps leading to the production of viruses continued at rapid speed and sooner than later, particles started form internally. Presumably, therefore, their number within the host cells increased even during eclipse (broken lines). If at this stage, the bacterial cells were to be ruptured, infective virions could be easily detected. The entire phase starting with adsorption and covering 'eclipse' is known as the latent period (*c*). At the end of this period the host cell membrane and wall were ruptured due to 'lysis from within'. Virtually all the infected cells were lysed at the same time as reflected in an abrupt increase in the number of virus particles (*d*). Thus the 'Lytic Cycle' is completed and took about 28 minutes.

In order to ensure that the virus particles are formed at the same time and that the progeny particles do not infect the uninfected host cells, the entire suspension is diluted many times with the bacterial medium. This results in sparse distribution of bacterial cells and prevents newly formed particles from infecting them. Such condition is known as One Step Condition and the multiplication curve as One Step Multiplication Curve.

nucleic acid into the host cell. It was known from earlier studies that only the nucleic acid portion of the phage entered the cell and that the entire phage nucleic acid molecule was necessary for infection

leading to lysis (Hershy and Chase 1952). Obviously, some mechanism must be operative which would on the one hand separate the nucleic acid from the sheath and, on the other hand, find a means of introducing it into the host cell.

Recent studies have brought out the details of the entire operation very clearly. It is now established that the bacteriophages are able to hydrolyze the mucopolypeptides of the *E. coli* cell wall, showing an enzymic activity similar to that of lysozyme. This phage lysozyme is synthesized during phage multiplication and the molecules of the enzyme remain attached to the tips of tail of the phages when these are released from the host cell. On adsorption this enzyme system is supposed to drill a hole through the bacterial cell wall through which the nucleic acid is injected into the cell.

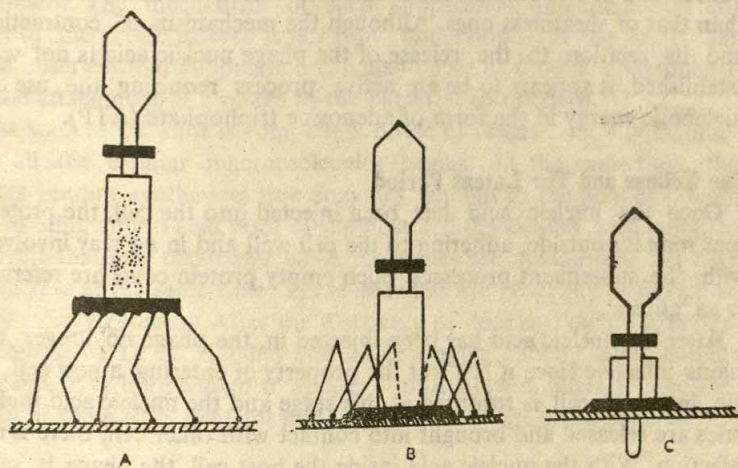


FIG. 5.5 Mechanism of penetration of bacteriophage T₄. The phages first get absorbed to the host cell surface by means of their tail fibres. Once absorbed, the phage presses down. The fibres gradually bend. In the meanwhile the contractile sheath covering the tail core contracts. The plate and the fibres are adjacent to the cell surface by now. The core penetrates through the cell wall, followed by release of the nucleic acid.

Electron micrographic studies show that the sheath around the coat of the tail retracts during this period. In a recent report Dr E. Kellenberger has described the process very beautifully (Kellenberger 1970). Accordingly, at first the tail fibers become bent, bringing the base plate of the phage in contact with the bacterial cell wall. The hexagonal plate looks like a star at that time. The tail sheath then contracts and the base plate is pushed up along the needle or the central

tubular part of the protein coat. The needle is pushed through the hole in the wall and the nucleic acid flows through the hollow centre of the tubular needle. Because of this action, the virion has been compared to a hypodermic syringe and the release of the nucleic acid is called *injection* (Fig. 5.5).

It is more or less definite that the tube of the *T-even* coliphages does not penetrate the bacterial plasma membrane. DNA of the phages has often been located on the membrane surface itself.

The contraction of the sheath is not essential for the release of the nucleic acid. Many mutants of *T-even* coliphages as well as small helical bacteriophages like M13 lack the sheath without any effect on the injection of their nucleic acids. The sheath, however, is known to increase the rate of penetration of the phage DNA. Generally, the rates of penetration of nucleic acid from sheathed varieties is many times more than that of sheathless ones. Although the mechanism of contraction and its relation to the release of the phage nucleic acid is not well established, it appears to be an active process requiring the use of metabolic energy in the form of adenosine triphosphate (ATP).

The Eclipse and The Latent Period

Once the nucleic acid has been injected into the cell, the protein coat remains outside, adhering to the cell wall and in no way involved with the subsequent processes. Such empty protein coats are referred to as 'ghosts'.

After the nucleic acid has been injected in, the phage no longer remains infective since it has lost the property of entering a new cell. If the bacterial cell is ruptured at this stage and the nucleic acid molecules are released and brought into contact with other cell, there is no infection. With the nucleic acid inside the host cell, the phage is said to be in the *eclipse* because no perceptible activity of the phage is noticeable. During this period the formative stage of new phage particles begins. The eclipse is said to be over when discernible viral activity in terms of assembly of new nucleic acid and protein molecules into the first infective virion is noted.

The two immediate effects of entry of the viral nucleic acid are: (1) immunity against infection by phages of the same type, though not of other types, and (2) suppression of all activity of the host cell.

The former process is mediated by the formation of specific enzyme repressors which act against multiplication of phages of the same type. The mechanism behind such immunity is not very well understood. It definitely has nothing to do with immunity in the sense of immunology based on antibody-antigen interactions since bacteria do not

possess immunological defences. However, the process is known to be gene controlled.

The phenomenon of suppression, on the other hand, involves the cessation of synthesis of all cellular DNA, RNA and proteins. Nevertheless, evidences suggest that during this phase synthesis of viral nucleic acid begins. Therefore, this stoppage is more qualitative than quantitative. It has been demonstrated that the enzyme already present at the time of infection continue to function, as do the ribosomal RNA molecules. The overall rate of protein synthesis does not appreciably change. It has been suggested that on infection, a profound rearrangement of all major macromolecular synthesis occurs. There is a shift from cellular to viral synthesis and the latter becomes gradually dominant. The mechanism of such suppression and the concurrent shift in metabolic pattern is not fully understood. However, it has been observed that suppression can also occur as a mere consequence to the attachment of a phage to the bacterial cell surface.

As soon as the shift is complete a phase of intense activity embracing all the cellular macromolecules begins. At the same time, the phage genome synthesizes new enzymes, foreign to the host cell, from the existing amino acid pool of the host cell. Evidences are there to indicate that new messenger RNA molecules are synthesized at a very rapid rate at the same time. These enzymes are generally referred to as the *Early Proteins*. After the discovery of deoxycytidylate hydroxymethylase by Dr A. Hershey and his colleagues, many such specific proteins have been shown to appear within a few minutes of infection. A list of these early proteins and their functions are given in Table 5.2

TABLE 5.2. CHARACTERISTICS OF SOME EARLY PROTEINS

| Name | Function |
|------------------------------|---|
| DNA polymerase | Synthesis of new DNA |
| Glucosyl transferase | Glucosylation of phage DNA |
| Deoxycytidine triphosphatase | Destruction of cytidine containing nucleotides |
| Deoxyribonuclease | Breakdown of cellular DNA |
| Structural proteins | Sealing of the cell wall orifice; Internal proteins |

The formation of early proteins initiates several specific operations. Some of these proteins seal off the orifice created on the cell wall due to penetration of the phage nucleic acid. The specific DNases depolymerise the DNA of the host cell and break it down into the constituent nucleotide units. Almost simultaneously, viral nucleic acid begins

to be synthesized out of the enlarged nucleotide pool created in the host cell. Often new and exclusive viral nucleotides are synthesized.

Synthesis of viral DNA is an extremely well regulated phenomenon. Apparently, it begins with unwinding of the double stranded DNA of the phage followed by synthesis of new nucleic acid molecules utilizing the precursors and energy resources of the host cell. The new DNA thus synthesized are constituted of two types of strands, one old, belonging to the parental viral nucleic acid and the other newly formed one synthesized using the former as the template. This type of replication is called semi-conservative replication. More and more viral nucleic acid molecules are synthesized in this fashion.

Apart from direct synthesis of DNA molecules, replication is also accompanied by breakage and reunion of the molecules. For instance, molecules belonging to one generation could exchange fragments with molecules of another generation, thereby creating new hybrid molecules. Such a behaviour gives ample scope for several types of genetic changes to occur subsequently, all within the same growth cycle.

The mechanism of regulation of these various phenomena associated with the process of viral replication is not fully understood. Evidently, these are controlled by the viral genome. For instance, it has been established that different regions in the genome of the bacteriophages *T*₄ (King 1968) and ϕ X174 regulate specific operations like the promotion of the synthesis of viral DNA and *m*-RNA molecules. However, the nature of the control process that initiates all these operations almost simultaneously, yet independently of each other, is still to be fully appreciated. The most profound and fundamental aspect of these phenomena is the gradual but definite establishment of dominance of the viral genome over that of the host bacterium. This dominance is complete and unique in the sense that the metabolic functions of the host are not stopped but rather are made subservient to the requirements of the invading virion.

After the synthesis of viral nucleic acid, and sometimes, along with it, a new type of proteins start appearing. These are called the *Late Proteins* and their formation is dependent upon the activities of the nucleic acid molecules formed afresh. Two types of the late proteins are identified in bacteriophages infecting *E. Coli*, namely, the viral coat proteins and the viral lysozymes.

Cost proteins constitute the majority portion of the total late proteins synthesized. Their synthesis begins with the formation of the new phage nucleic acid and continues throughout the eclipse period. Isotopic studies carried out in recent years reveal that, initially, polypeptide chains are synthesized. These chains then go to form subunits

which are assembled into monomers or oligomers or polymers that go to form larger structures like capsomeres. Later on, these larger structures are assembled into virions. Evidences indicate that at each level of formation, a 'pool' of the ingredients is formed. From these subunits are withdrawn at random to form more complex structures.

Specific antigenic properties of the coat proteins are acquired at the initial stages of these developments. Enzymes with marked similarity to lysozyme are synthesized in cells infected with bacteriophages at the later stages of viral replication. These are definitely caused by the expression of viral genes and in all probability, are synthesised prior to the assembly of the protein and nucleic acid components into new virions.

Maturation

After the various components of the progeny virions are formed, their assembly into mature or complete particles begins. This period of assembly is called *maturation*. This process is evidently controlled by the viral genome. It has been postulated by Drs J.D. Watson and F.C. Crick (1972) that the assembly of the preformed parts into their respective virions is essentially a process of condensation and that the shape and design of a viral capsid is determined by the specific bonding properties of its identical capsomeres. This basic tenet appears to hold good for all varieties of host-virion systems. A detailed analysis of the process involving different viruses indicates that maturation is a sequential event, in which each step requires and recognizes the previous one. Each step is linked to the phases of a crystallization process in which the product has a lower level of free energy than the unassembled parts.

Maturation of *T-even* bacteriophages is a complex process and involves the assembly of many components. Formation of head and tail begin separately, though not simultaneously. The first step towards head assembly is the condensation of individual viral DNA molecules into crystalline particles which resemble phage heads and are surrounded by a thin membrane at the same time being different from a regular capsid. Condensation of DNA is facilitated presumably by an interaction of the DNA with a *condensing principle*, probably the internal protein or the cationic polyamines. The capsid monomers then aggregated around the DNA condensate and form the phage heads. To be stable, it is imperative that these be immediately attached to the tails.

Tail assembly starts in the meanwhile. Base plates are formed first, and initiate the formation of the core tube. At this stage, the partially

formed tail is attached to the head. After attachment, sheath subunits are started to be formed around the core starting from the base plate. Fiber assembly, which is also gene controlled, is completed separately from several subunits. After formation these are attached to the fibreless particles in a sequential manner. The attachment of fibres to their proper position in the tail are dependent upon antigenic properties of the tail fibres (Fig. 5.6).

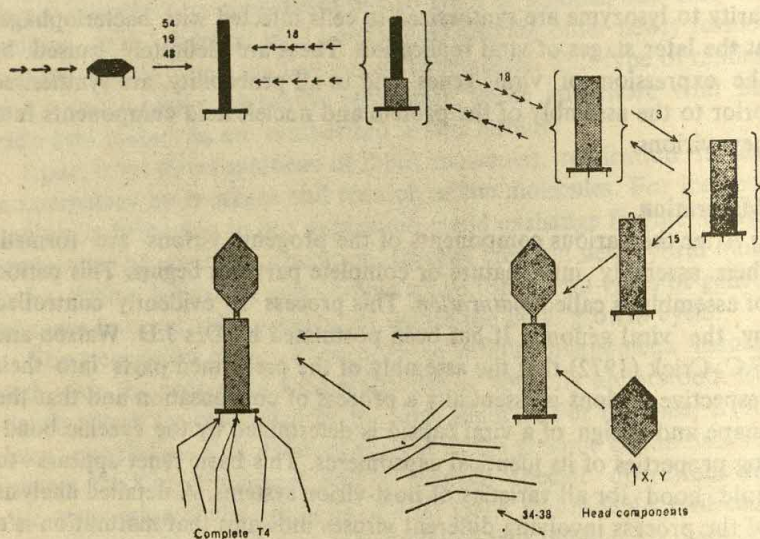


FIG. 5.6. T_4 genes involved in assembly of bacteriophage T_4 (ref. Fig. 5.2). (After Edgar and Lielausis 1968).

Hundreds of new phages are thus formed. The excess, unused components remain as such. The formation of mature virions inside the host cell signals the end of the eclipse period. This period is the interval between injection of viral nucleic acid and the first appearance of new virions. This duration in the case of coliphage T_2 is about 12 minutes. The assembly of virions continues for some time after the eclipse. Generally it is completed as soon as the necessary metabolites in the host cell are completely used up, and is signalled by the destruction of the bacterial cell or its lysis. The time taken from initial induction of the nucleic acid to the inception of cell wall rupture is called the *Latent period*. For T_2 phages it is about 18 minutes.

Lysis and Release

At the end of the latent period the host cell wall ruptures or bursts and the virions are released (Fig. 5.7). The host cell is said to have

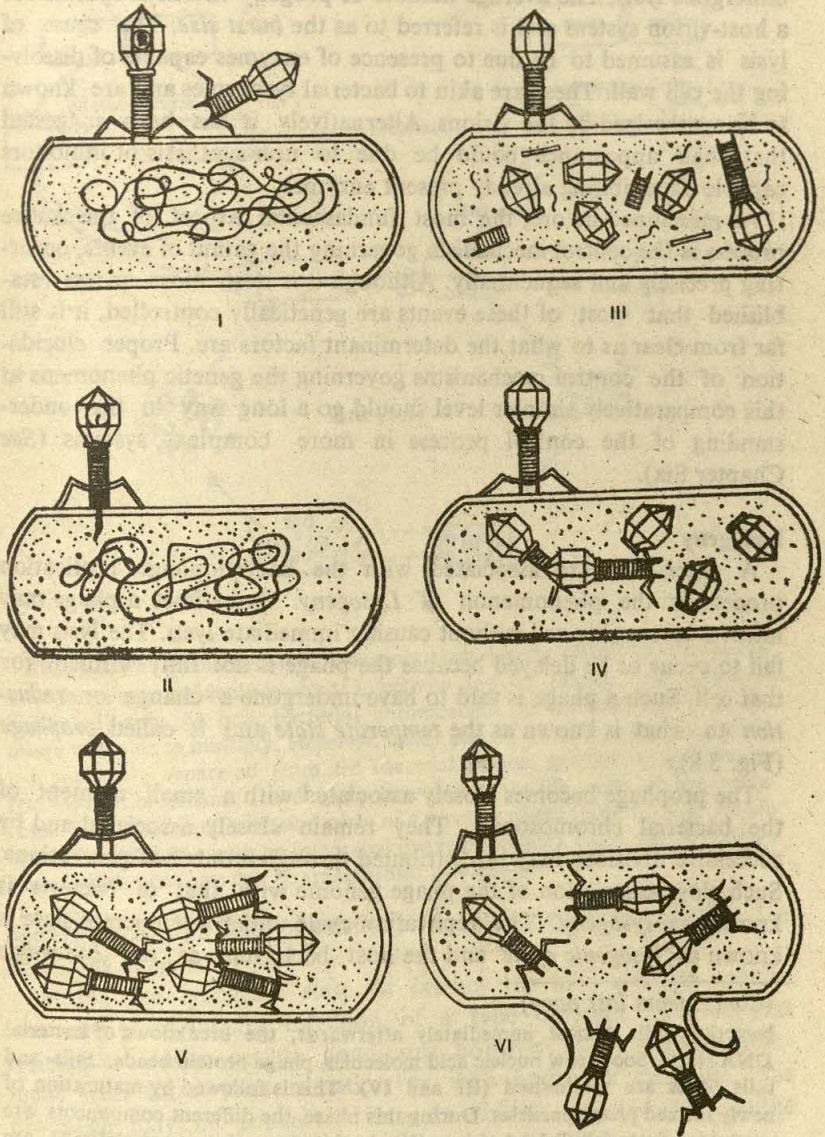


FIG. 5.7. The lytic cycle. Formation of virulent phages in a susceptible bacterium. The virus particles are attached onto the cell surface the tail fibres (I). Within a short time the bacterial wall and membrane are perforated by enzymatic action at the tail. Almost simultaneously the nucleic acid of the phage enters the host bacterium (II). The protein coat remains attached to the
(Contd. on next page)

undergone *lysis*. The average number of progeny virions is specific for a host-virion system and is referred to as the *burst size*. The cause of lysis is assumed to be due to presence of enzymes capable of dissolving the cell wall. These are akin to bacterial lysozymes and are known to be synthesized by the virions. Alternatively it has been suggested that their appearance could be due to non-synthesis of inhibitors capable of inhibiting already present enzymes.

As mentioned before, the most fundamental aspect of the entire process is the control mechanism governing the gamut of events, occurring precisely and sequentially. Although it is now more or less established that most of these events are genetically controlled, it is still far from clear as to what the determinant factors are. Proper elucidation of the control mechanisms governing the genetic phenomena at this comparatively simpler level should go a long way in the understanding of the control process in more complex systems (See Chapter Six).

Lysogeny

A special feature associated with the bacteriophage replication process is the phenomenon of *Lysogeny*. Sometimes a phage may infect a susceptible cell without causing immediate lysis. The lysis may fail to occur or be delayed because the phage is not fully virulent for that cell. Such a phage is said to have undergone a change or *reduction* to what is known as the *temperate state* and is called *prophage* (Fig. 5.8).

The prophage becomes closely associated with a small segment of the bacterial chromosomes. They remain closely associated and by successive divisions may be distributed through number of generations. Such close association of the phage genome with that of bacteria is known as *lysogeny*. The replication cycle under a temperate state is known as '*lysogenic cycle*' and the host bacterium as the *Lysogenic*

(Contd. from last page)

bacterial wall. Almost immediately afterwards, the breakdown of bacterial DNA starts. Soon, new nucleic acid molecules, phage protein heads, tails and tails fibres are synthesised (III and IV). This is followed by maturation of newly formed phage particles. During this phase, the different components are assembled into full-fledged virions (V). At this stage, if the bacterial cells are ruptured, complete newly formed virions may be seen. The assembly starts after about 16 minutes of adsorption. This takes about 12 minutes to be fully completed. After this the cell wall and the membrane are lysed from within (VI). The number of phage particles formed before lysis is characteristic for a particular virion and is known as the '*burst size*'. The whole cycle takes about half an hour to be completed.

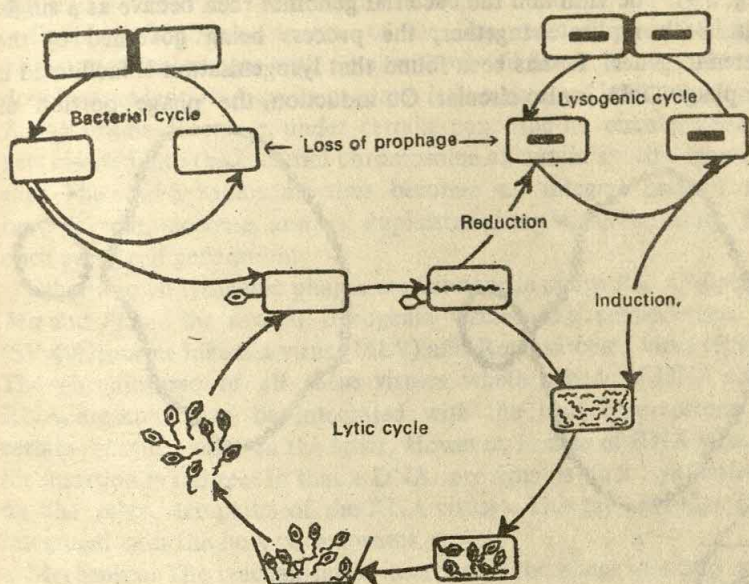


FIG. 5.8. The phenomenon of lysogenization. Under certain circumstances, a virulent phage suddenly loses its infectivity and its nucleic acid core gets integrated with the host bacterial chromosome. In an integrated state, the phage is unable to multiply. However, under certain conditions these inactivated phage are separated from the bacterial genome, multiply and ultimately lyse the host bacterium. The diagram here depicts the life cycle of the bacterium (top left), multiplication cycle of a virulent phage (bottom), and the life cycle of a bacterium with an integrated phage (top right). Note the conditions under which one form can be transformed into another.

Bacterium. Such a state of existence in a higher plant or animal cell is known as *latency*.

During the lysogenic state or latency the host cell may acquire immunity from further infection of a type different from natural resistance. Under certain conditions, however, as for example treatment with ultra-violet radiation or with hydrogen peroxide, the prophage may be converted into its virulent state to undergo the vegetative lytic cycle. Such conversions are called *induction*.

In recent years details of several aspects of this phenomenon have been established. We now know that the prophage does not have a fixed point of attachment on its genome. Rather, it gets attached as a circular loop to the cyclic bacterial DNA. As a result, the latter opens up and the viral DNA gets linearly inserted into the bacterial genome

(Fig. 5.9). The viral and the bacterial genomes then behave as a single unit. Both replicate together, the process being governed by the bacterial genes. It has been found that lysogenisation is facilitated if the phage DNA is also circular. On induction, the phage portion of

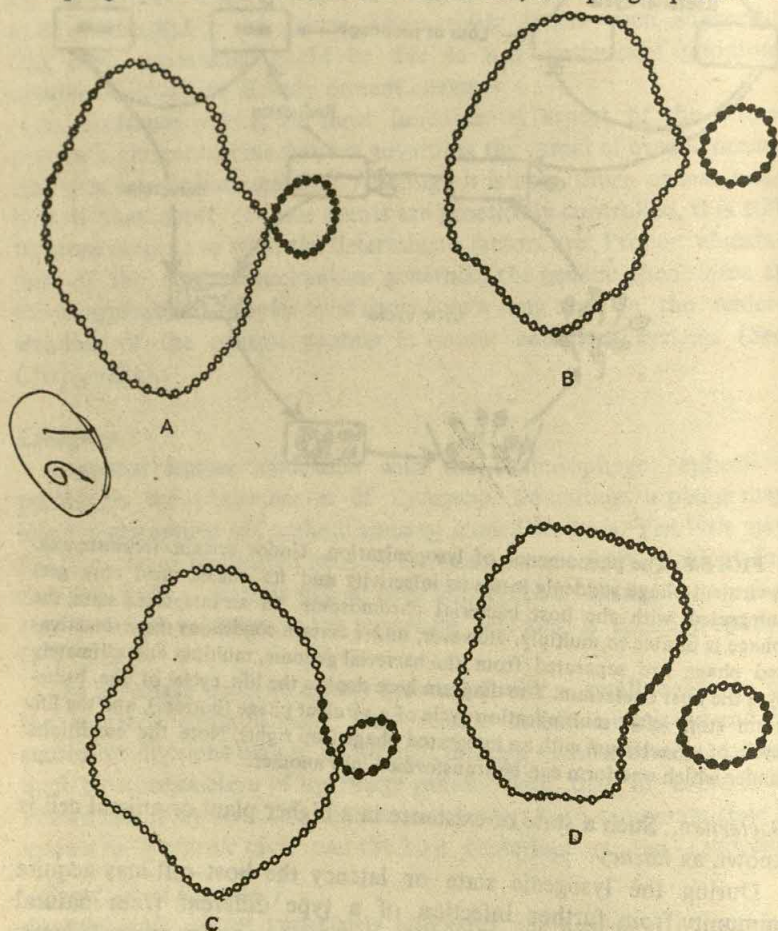


FIG. 5.9. Mechanism of separation of phage DNA from its integrated state. Under normal circumstances the separation is normal (A and B). However, under abnormal conditions, the separation is incomplete (C and D). In these cases there is an intricate exchange between the bacterial and the viral genomes. The separated viral genome now possesses a tiny fragment of the bacterial chromosome. This mechanism explains both transduction and lysogenic conversion. In the former situation, the separated phage with bacterial DNA gets integrated with a new bacterial chromosome and alters it. With the latter, it is the viral DNA that alters the characteristics of the host bacterium.

this unit is selectively dissociated in a reverse manner.

Coliphage lambda (λ) is one of the important lysogenic phages. These are binal phages with a tail without tail fibers. It has a circular double stranded DNA as its genome. It is capable of lytic infection of *E. coli* strains. However, under certain condition its circular genome gets inserted into the bacterial chromosome at certain specific insertion sites. The viral chromosome thus becomes an integral part of the bacterial chromosome and is duplicated along with the former, just once every cell generation.

Other known lysogenic phages are Salmonella phage P_{22} , Coliphage Mu and Pi and the several oncogenic viruses like simian virus 40 (SV 40), murine leukemia virus (MLV) and Rous sarcoma virus (RSV). The chromosome of all these viruses, whether made of DNA or of RNA, are known to be integrated with the host chromosome at certain specific sites on the latter. However, in case of RNA viruses, the insertion is indirect in that a DNA provirus is first synthesized, on the RNA template of the RNA viruses. This provirus then gets integrated with the host chromosome.

Mechanism. The mechanism of insertion of the phage genome and its manner of segregation have also been elucidated to a considerable extent. For instance, it has been brought out that in both the host bacterium and the phage genome (*i.e.* DNA) there are certain regions where the two strands cease to be complementary. Consequently hydrogen bonding and other attendant interactions between the two strands are absent. The portion of the DNA strands at these regions tend to loop out and can be seen under the electron microscope. These regions are called *Hetero-Duplexes*. This suggests that the integrating phage genome (DNA) and the host genome need not be complementary to form an association (See Stent 1971).

It has been also discovered that there are specific DNA sequences, usually less than 2000 base pair long, on both the genome of *E. coli* and its phages, which get frequently translocated from one to another site. Such short sequences have been termed insertion sequences or *insertosomes*. In the coliphage genome, these insertion sequences have been found to be associated with a number of specific marker genes. In fact, the insertosomes are present on both sides of a gene. It is now thought that the insertion sequences get dissociated from the genome and carry with it one gene or the other. The insertion sequences can associate with the host genome also and thereby are responsible for integrating a phage or its portion.

Lysogeny indicates a close evolutionary link between phages and bacteria. Complete association of the nucleic acids, generally DNA, of

the two indicates the presence of a high degree of homology at the level of this macromolecule. Also, such integrated association has additional survival value for the phage since it makes their parasitism on the host total and all embracing.

Lysogenic incorporation of the viral genome into the bacterial chromosome gives some clue as to how viruses might have originated as detached portions of the chromosomes of bacteria or some other host cells. It has been argued that such detachment could be taken as an attempt towards independent existence. Due to changed circumstances this could not succeed and reincorporation into their parent chromosomes followed.

Putting forth an alternative view Prof. M Pollock has suggested that the genetic material of bacterial cells (and may be other primitive organisations) were themselves mosaics that have developed and grown by incorporating a variety of smaller particles. This would mean that particles with limited genetic potentialities arose independently and bacterial genome (and ultimately that of the eukaryot) were formed by a number of such particles aggregating to form the chromosomes.

It is premature to say which of these process actually occurred. As pointed out by Prof J.A.V. Butler, (See Butler 1970), both these process actually might have occurred. Accordingly, the bacterial chromosomes could be regarded as being in a dynamic state with components seggregating and reuniting in certain fixed frequencies. It has been suggested that such equilibriated status might exist in other type of chromosomes.

REPLICATION OF SOME OTHER VIRUSES

The process of replication of other viruses are basically similar to the situation obtaining in case of bacteriophage of the T series. However, modifications and variations with relation to different host-virion systems are there. We will consider some of these systems briefly in the following paragraphs.

Replication in Bacteriophages with Single Stranded DNA

The small bacteriophage ϕ X174 is known to have single stranded DNA. Its discoverer, Dr R.L. Sinsheimer, has also reported its mode of replication (Sinsheimer and Denhardt 1965). In this case the first thing that happens after penetration is the formation of a complementary stranded form, known as the *replicative form* (RF). The RF is utilised for the synthesis of linear messenger RNA molecules and

ultimately, the various early (enzyme) proteins. Besides, the RF also arranges for its own replication. DNA replication occurs almost immediately and ceases after the required number of viral DNA molecules are formed. Rest of the procedure is similar to the process operative in the case of coliphage T_2 (Fig. 5.10).

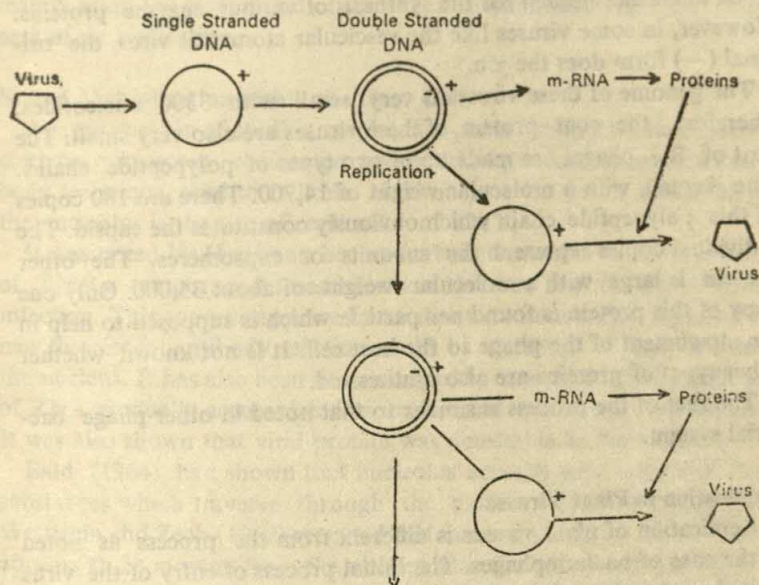


FIG. 5.10. Replication in bacteriophage $\phi X174$. This phage has single stranded DNA. Its replication pattern is, therefore, a bit different from those of other bacteriophages. In this case, after penetration the nucleic acid molecule synthesizes a complementary strand called the replicative form (RF). The replicative form in its turn synthesises other DNA molecules and the protein sheath components. However, out of many copies of the RF only one is used for DNA synthesis. The rest are utilized for the synthesis of messenger RNA and proteins. The bacterial metabolic processes are shut off as usual.

In recent years the mechanism of genetical control over $\phi \times 174$ replication has been worked out (See Chapter Six.)

Replication in RNA Phages

RNA phages like f_2 , QB and R_{17} are also small particles. After infection and penetration, the single stranded RNA molecules act as template and direct the synthesis of replicative forms of RNA. This synthesis is mediated by the enzyme RNA replicase produced in the host cell after viral infection. This form obviously is double stranded

and subsequently directs the synthesis of more and more viral nucleic acid. Prior to the formation of the replicative form, some of the original virus RNA also start the synthesis of enzyme proteins after getting attached to the host cell ribosomes. Generally, the (+) or the newly formed strand (replicative form) of RNA synthesises messenger RNA molecules needed for the synthesis of various enzyme proteins. However, in some viruses like the vesicular stomatitis virus, the original (-) form does the job.

The genome of these viruses is very small with 3300 nucleotides. Therefore, the coat protein of these viruses are also very small. The coat of R₁₇ phages are made up of two types of polypeptide chains. One is small, with a molecular weight of 14,700. There are 180 copies of this 1 polypeptide chain which obviously constitutes the capsid. The individual copies represent the subunits or capsomeres. The other protein is large with a molecular weight of about 35,000. Only one copy of this protein is found per particle which is supposed to help in the attachment of the phage to the host cell. It is not known whether other types of proteins are also synthesized.

The rest of the process is similar to that noted in other phage bacterial system.

Replication in Plant Viruses

Replication of plant viruses is different from the process as noted in the case of bacteriophages. The initial process of entry of the virus particle into the host cell has been found to involve entry of virus particles *in toto*. There is no prior separation of the protein coat from the nucleic acid core. The process of initial entry thus is analogous to the process of swallowing or *pinocytosis*. However, often injury on the surface of the host cell facilitates infection. A major number of plant viruses are vector transmitted. The mouth parts of the vectors, such as the stylets of aphids, play an important role in injecting the virus particles into the host cell.

Another interesting feature is that plant viruses do not generally replicate in the cells into which they are initially injected. Rather they are transported through plasmodesmata from cell to cell till they reach the site for multiplication. Often they form cellular inclusion bodies, as was noted in the case of TMV infected tobacco plants (Easu 1941). Sometimes specific sites of infection are needed for successful multiplication. For example it was observed that barley yellow dwarf virus are phloem limited and would multiply only in phloem sieve element (Easu 1957). In some cases, multiplication and translocation of virus particles are carried out simultaneously.

Our information concerning multiplication of viruses in plants are based mostly upon investigation carried with tobacco mosaic virus (TMV). Important data have also been collected on potato yellow dwarf virus, tomato spotted wilt virus, sugar beet yellow virus and turnip yellow mosaic virus. The investigation carried out centred mainly upon the elucidation of the site of multiplication and the process of multiplication.

Site of Multiplication within the Host Cell

A large number of studies, employing phase contrast microscopy, electron microscopy, radioactive traces techniques and fluorescent antibody technique, tend to indicate that nucleus or more specifically, the nucleolus is the site of replication of TMV.

It was noted by Hirai and his co-workers that soon after injection of TMV a temporary fluorescence was observed near the site of virus infection. This soon disappeared and about six hours after injection a new fluorescent antibody reaction could be detected in and around the nucleus. It has also been demonstrated by them that a large amount of RNA gradually accumulated as nucleic and cytoplasmic inclusions. It was also shown that viral protein was detectable in the nucleus.

Bald (1964) had shown that nucleolus actually gave off RNA like substances which traverse through the nucleus into the cytoplasm. Wettstein and Zech (1962) reported that in some strains of TMV, RNA appears to be moving from the nucleus into the cytoplasm. He claimed the occurrence of "an elaborate system of cytoplasmic channels" through which the RNA came out of the nucleus. He also observed the presence of envelop line surrounding around the RNA. Smith and Schlegel (1965) found that RNA directed RNA synthesis, meaning viral RNA synthesis in TMV occurred in the nucleolus.

Similar conclusion has also been reached by Reddi who concludes that the three chief events following the establishment of TMV particles in the host cell nucleus are:

- (1) Viral RNA controls host cell metabolism;
- (2) Continuous RNA dependent RNA synthesis; and
- (3) Production of capsid proteins.

Apparently the arrangement of complete particles into inclusion bodies starts occurring in the nucleus but is often completed in the cytoplasm.

The Process of Replication in TMV

Although direct evidences illustrating the sequential occurrence of the process of replication in TMV are not as yet available, a reasona-

bly clear picture does emerge out of the various studies mentioned earlier. These could be summarised as follows:

(1) the virus particles make entry into the host tissue directly, either through the stomatal openings or through microscopic injuries on the surface.

(2) the particles enter the cell *in toto*, possibly by means of a mechanism analogous to pinocytosis or phagocytosis.

(3) the particles remain intact and may pass on from one cell to the next via the plasmodesmata.

(4) Some of the particles invade the nucleus through the nuclear membrane.

(5) inside the nucleus, the nucleolus becomes the main centre of activity.

(6) the metabolic functions of the host cell nucleus comes under the control of the viral nucleic acid.

(7) RNA dependent RNA synthesis occurs in the nucleus.

(8) viral capsid proteins are also synthesised within the nucleus.

(9) Assembly of protein subunits and the nucleic acid starts within the nucleus. However, incomplete particles may also come out of the nucleus into the cytoplasm and assembly completed there.

The above scheme seems to be operative in the case of other plant viruses as well. In tomato spotted wilt virus (TSWV) the process has been found to be similar except that the process of assembly probably occurs in association with the endoplasmic reticulum. In sugar beet yellow virus (SBYV), vesicles are reported to develop around newly synthesised nucleic acid molecules. These vesicles remain within the nucleus and particle assembly occurs within them.

Assembly of the TMV Particle

A fundamental aspect in viral replication concerns the manner in which protein subunits arrange themselves around the nucleic acid core into the complete capsids. Although it is known that TMV capsid has a helical configuration, the precise manner in which the capsid subunits arrange themselves into the full-fledged protein coat is not yet fully known. However, in an interesting paper Butler and King (1972) have proposed a mode of such assembly.

According to them the protein subunits tend to form regular discs under certain ambient conditions (alkaline pH and low ionic strength). Once a disc is formed the assembly of protein subunits and nucleic acid into a complete particle starts. Certain specific sequences of nucleotides at one end of the TMV-RNA interacts with one disc. This interaction reorients the alignment of the disc in such a manner that

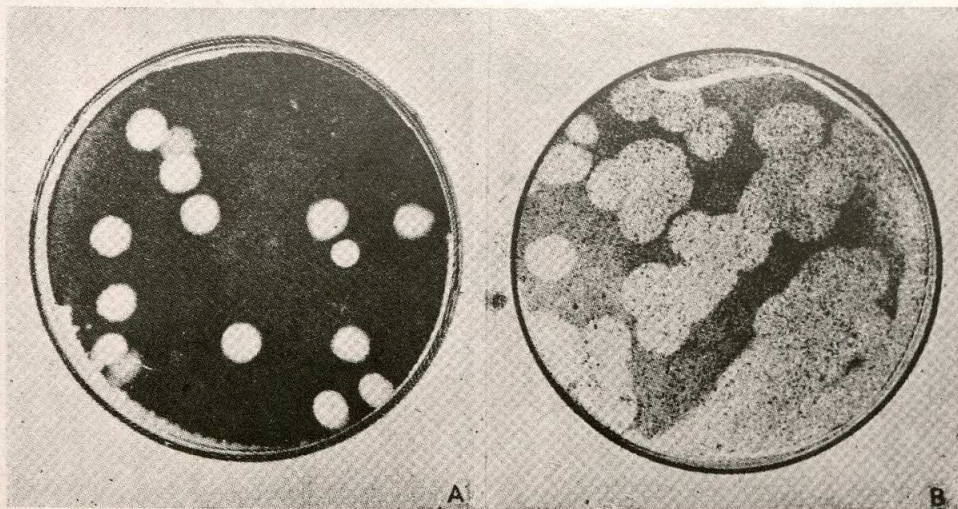


Plate I. Plaques of cyanophage AC-I on *Anacystis nidulans* (A) and *Chroococcus minor* (B). The differences in the morphology of the two plaque types are quite obvious. (Courtesy Dr G.S. Venkata Raman and B.D. Kaushik)



Plate II. Chorio-allantoic membrane of chick embryo infected by vaccinia virus. Note the small but prominent whitish patches representing virus colonies.

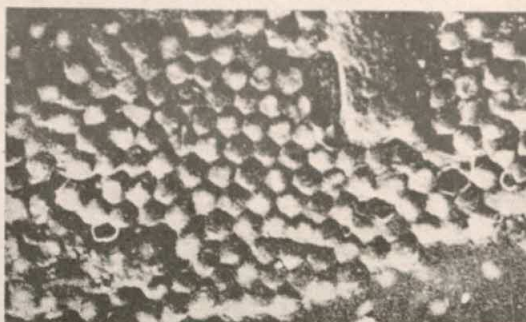


Plate III. Light micrograph (bottom) and Electron micrograph (top) of a crystal of *Tipula* iridescent virus bringing out clearly the difference in resolution.

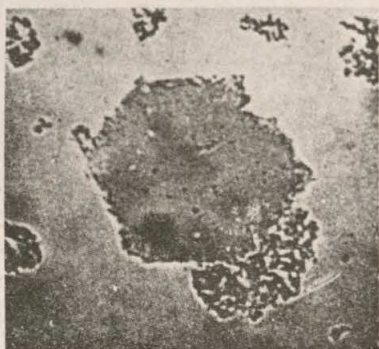


Plate IV. Bacteriophage T_2 DNA after release from the head ($\times 100,000$).

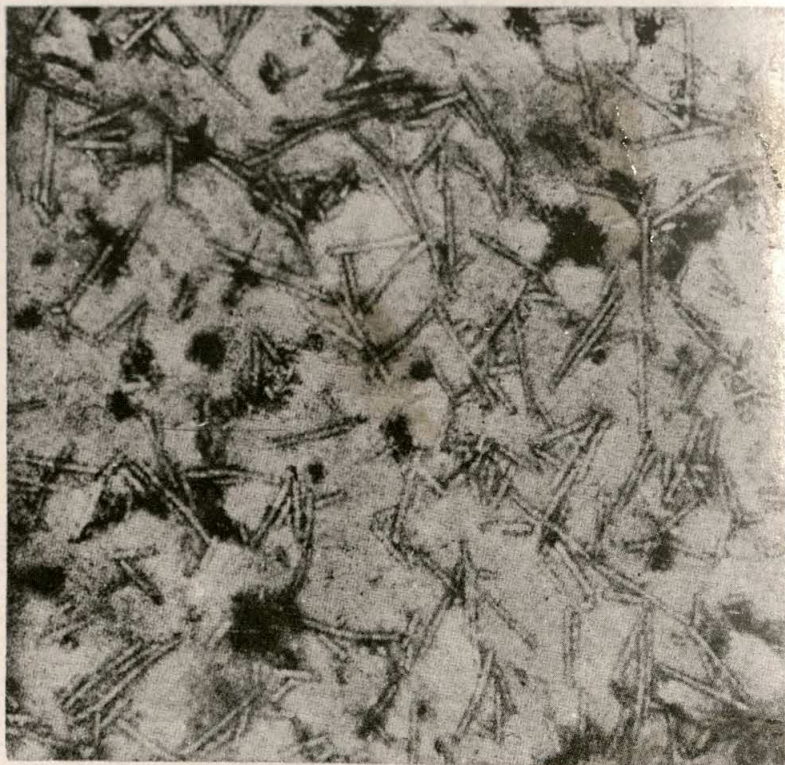


Plate V. Isolated crystals of a strain of tobacco mosaic virus $\times 42,000$.
(*Courtesy Dr Minati Ray Choudhury and Dr Anupam Varma.*)

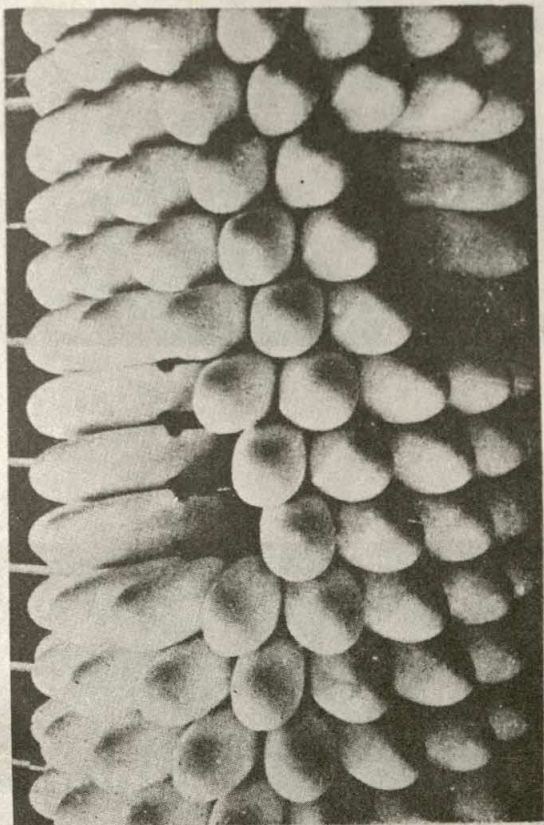


Plate VI. Photograph of a portion of a model of tobacco mosaic virus showing the protein sub-units arranged in a helix.

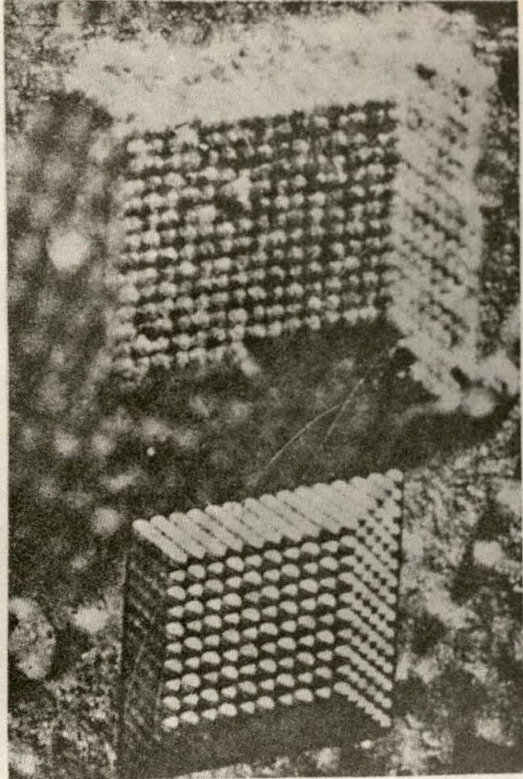


Plate VII. Electron micrograph of a crystal of tobacco necrosis virus. Note the shape of the crystal, which is typically rhombic. A model crystal of rhombic type has been reproduced below for comparison.

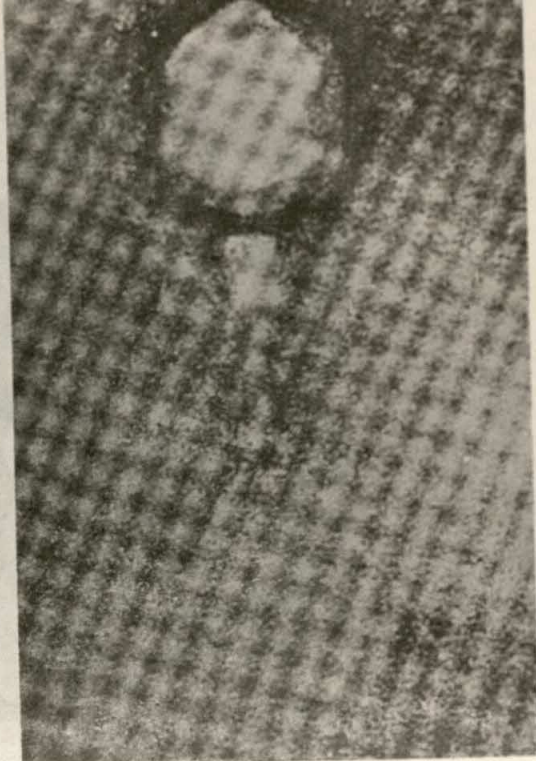


Plate VIII. Isolated particles of T₁ bacteriophage treated with hydrogen peroxide. Note the position of the retracted tail sheath and tail fibres ($\times 391,500$).

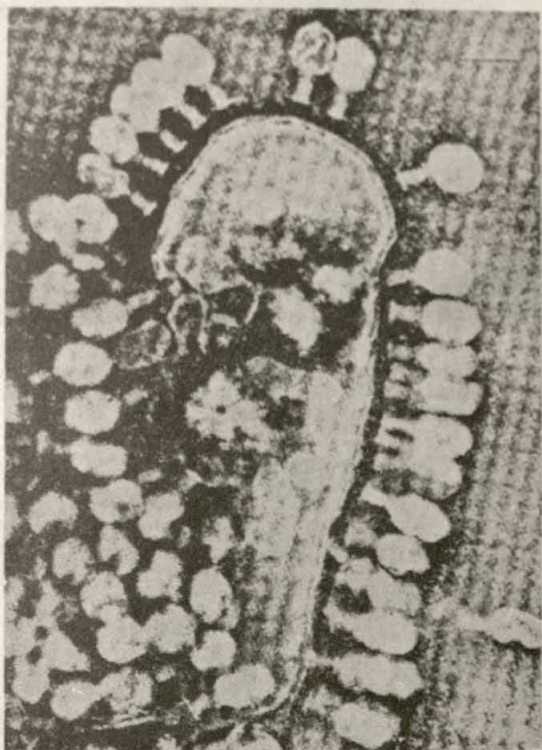


Plate IX. Electron micrograph showing bacteriophage T_4 particles adhering to the surface of the bacterium *Escherichia coli*.

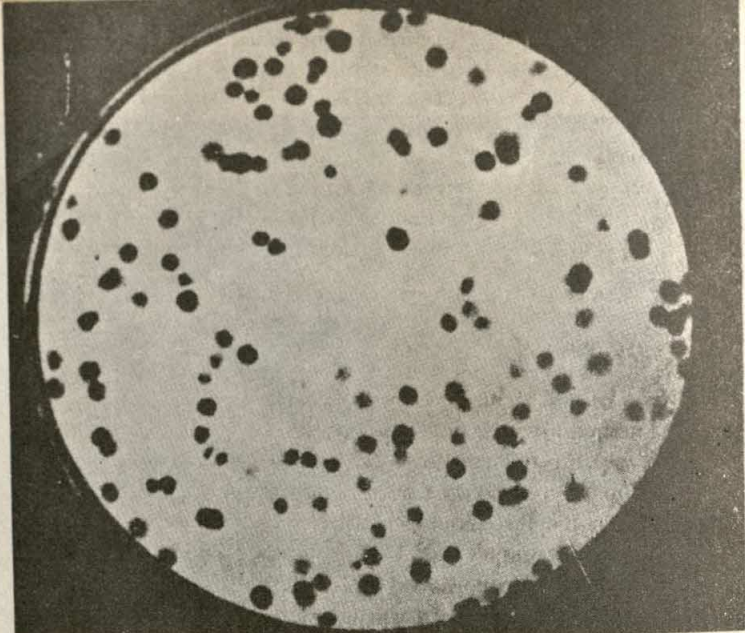


Plate X. Plaques formed by coliphage T_1 mutants on bacterial lawns. Note the difference between well formed (rapidly lysis) plaques and relatively ill defined wild type plaques (after Benzer 1956).

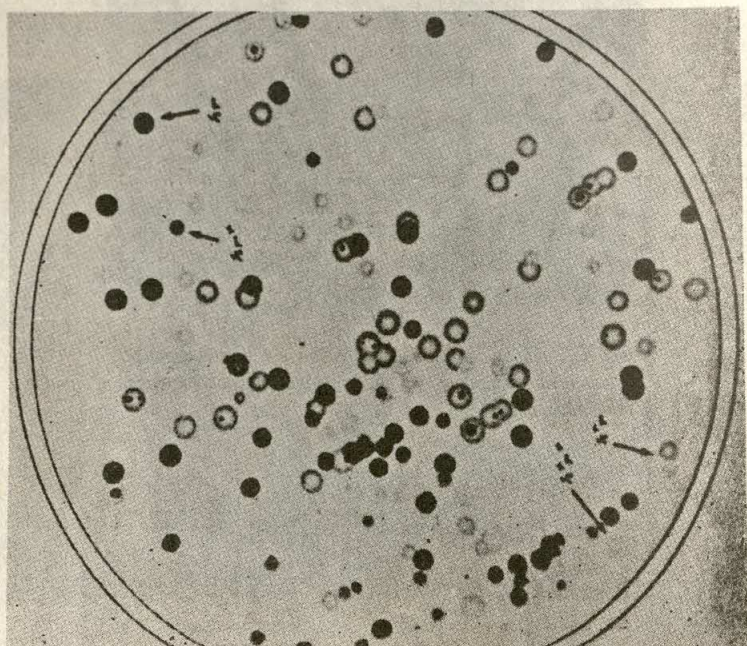


Plate XI. Recombination in bacteriophage T_2 . Note the development of four types of plaques on the bacterial lawns, viz, large-clear (T_2hr^-), small turbid ($T_2h^+r^+$), small clear ($T_2h^+r^-$) and large turbid (T_2hr^+). The first two are the parental and the others recombinant types. (after Benzer 1955).

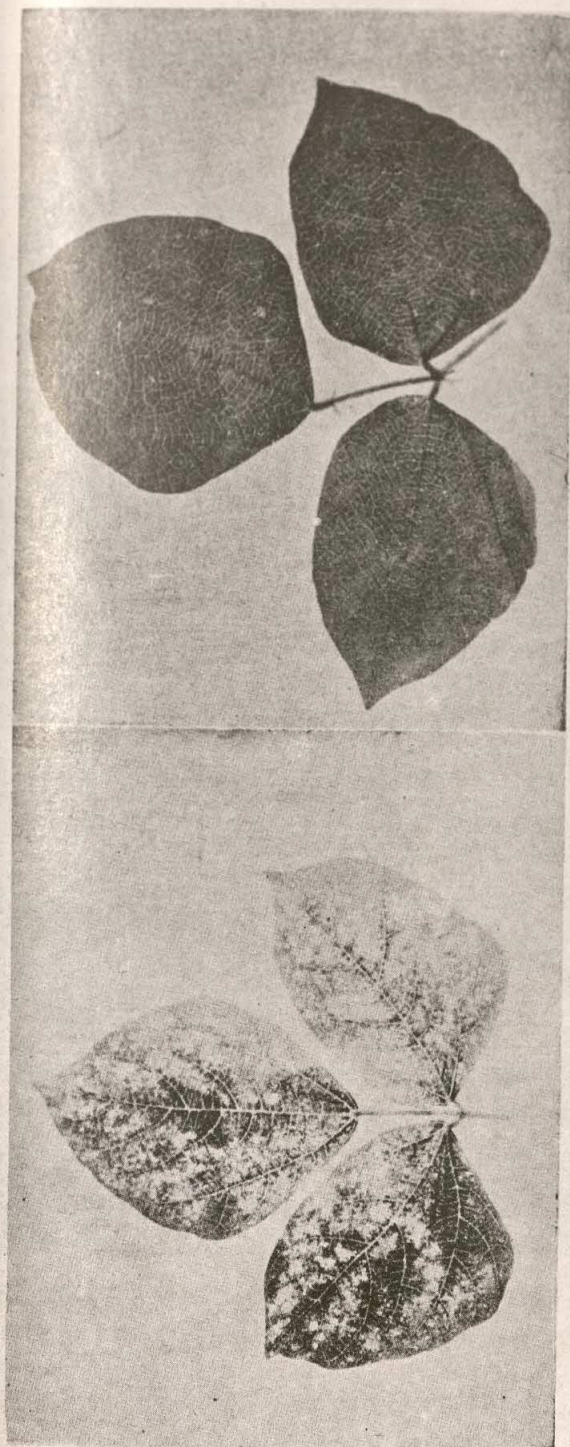


Plate XII. Photographs of pumpkin leaves infected by mung bean mosaic (left). Observe the changes from a normal leaf (right). (*Courtesy*. Miss Parveen Kaur).

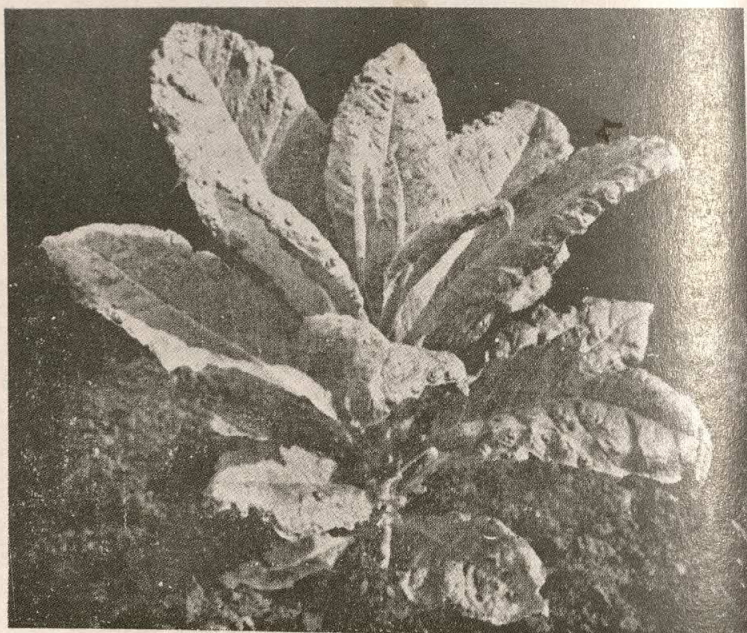


Plate XIII. Tobacco plant infected by TMV. Note the characteristic molting symptoms.



Plate XIV. Transverse section of a mung leaf infected by mung bean mosaic virus showing the presence of virus crystals inside the host cell. (*Courtesy Miss Parveen Kaur*).

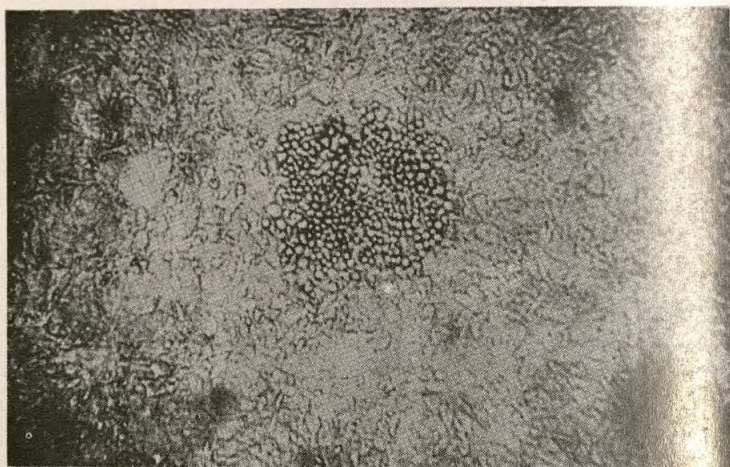


Plate XV. Cell transformation. Some liver cells infected by Hepatitis virus become morphologically different. (Compare with normal liver cells of plate XVI.)

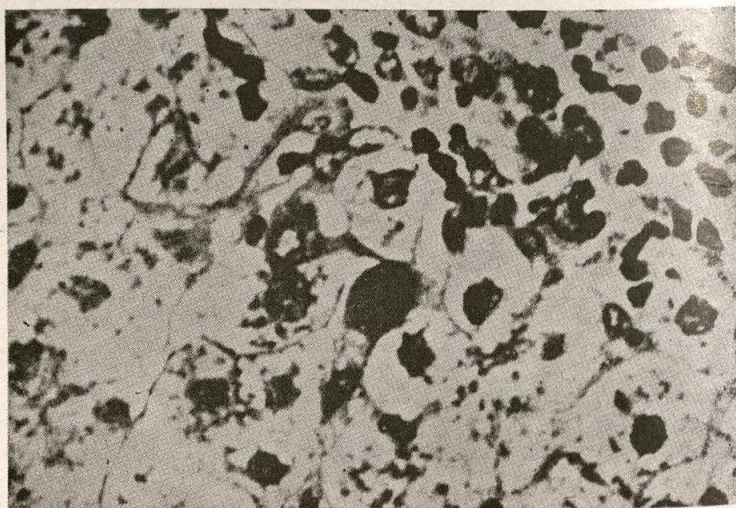


Plate XVI. Councilman bodies. Note the black dots inside liver cells infected by hepatitis cells. (after Sriramachary and Mahadevan 1974.)

the disc now appears to be two halves of a helix. Successive alignment of such disc turned helical capsid units ultimately leads to the formation of the helical rod of the TMV particle. This view, however, is not sufficiently evidenced and is often disputed (see Richards and Williams 1972).

Satellite Virus and its Role in Plant Virus Replication

Sometimes one virus may be completely dependent upon the assistance of another virus in the same cell to enable it to successfully replicate. Such a totally dependent virus is called the *satellite virus*. The virus which allows the satellite virus to successfully infect and replicate is called the *activator virus* (Kassanis 1967).

Tobacco necrosis virus (TNV) is one such activator virus. A satellite virus (SV) was reported to be invariably associated with TNV (Kassanis 1962). The satellite virus of TNV is a small (size 17 nm) RNA containing virus which cannot multiply on its own while TNV can. However, in presence of TNV, SV also multiplies. It contains RNA which is capable of coding for the SV coat protein. Since the SV-RNA can code for the coat protein, the help of the activator TNV is not required for that purpose. The exact nature of the help that SV must require is not clear as yet.

Tobacco rattle virus provides another example of satellitism. This virus occurs in two particle sizes, one large and the other small. The larger particles were shown to be incapable of replicating without the smaller ones. However, some virologists are reluctant to consider TRV as an example of satellitism because they point out that the two types found are related serologically. Tobacco necrosis virus (TNV) and its satellite virus (SV), however, are serologically unrelated.

REPLICATION IN ANIMAL VIRUSES

The process of replication has been investigated in several members belonging to different groups of vertebrate viruses. It has been noted that in spite of the wide variety in the details of the respective processes, certain common steps in the growth cycle of these viruses is discernible (Waterson 1968).

The first step is the entry of the virus into the host cell. The contact of an animal virus particle with the cell surface and its subsequent entry does not appear to be very specific since there is no evidence of specific receptor site on the host cell. The entry occurs by ingestion of the virus particle by the host cell *in toto*. Inside the cell, the particles are not free but rather are contained in a vesicle. Such process of

injection is called *phagocytosis*.

After entry into the cell, the virus is said to be in eclipse for a period referred to as the latent period. During this phase practically no infective virus can be detected in or recovered from the cell. The duration of this phase is longer in viruses with double stranded nucleic acid than for those with single stranded nucleic acid (Tamm and Egger 1965); for instance for adenovirus the duration may be as long as 12-13 hours but for western equine encephalitis (WEE) virus it is only for about 90 minutes. During this period serological properties and haemagglutinin activity in influenza virus is reported to be lost.

In general, the principal process going on during the latent period is the production of various 'early' proteins which are mostly enzymic. These enzymes are needed for the synthesis of the various viral constituents and are not themselves incorporated into virions.

After the formation of early proteins is completed, syntheses of viral nucleic acids and capsid protein units start. This is followed by the assembly of the individual components into full-fledged nucleocapsid particles. The time taken for completion of the process, as also the details of the process vary considerably from one host-virion system to another.

On maturation the new virus particles are released into the surrounding environment of the host cell. The mode of release and the quantum of release appears to vary widely from one animal virus group to another. In general, it has been suggested that there are two main ways by which viruses are released from their host cells. These are (1) by destruction of the cell or lysis and (2) by budding from the cell surface. Sometimes, as in the case of herpes group of viruses, there is no release into the environment. Rather, the particles are passed on from one cell to the next as is also observed with plant viruses.

An interesting mode of liberation is found in some myxoviruses, such as the influenza virus. These viruses mature at the cell surface and incorporate host cell membrane material. Thus they are capable of adhering to the cell membrane in a specific manner which facilitates their movement through the membrane. Once they come to lie on the outer surface, they are dissociated from the cell membrane by the activity of the enzyme neuraminidase (Seto and Rott 1966). The lipid component of the enveloped animal viruses also play an important role in the release of mature virus particles.

Growth cycles of large number of animal viruses have been worked out in recent years (see Martin 1967, Rott and Scholtisseic 1967; Green 1966). Some viruses have been studied in more detail than

others. Naturally, therefore, the view obtained is a composite one, individual aspects being drawn from different examples. We shall now consider in general the growth cycles exhibited by DNA and RNA containing animal viruses.

While the growth patterns exhibited by DNA viruses and by RNA viruses are generally the same, there are several important differences between the two. These are:

(1) RNA viruses multiply in the cytoplasm but all DNA viruses except the pox viruses do so in the nucleus.

(2) Growth cycle is longer in the case of RNA viruses than in DNA viruses.

(3) DNA viruses produce, during their growth cycle, viral RNA molecules whereas only a few RNA viruses are capable of synthesising DNA molecules.

Growth Cycle of DNA Viruses

The stages involved in the multiplication of DNA virus are as follows (Green 1966):

(1) Attachment and entry of the virus with subsequent transport to the site where replication will occur.

(2) Undressing of the particle and release of DNA.

(3) Transcription of viral messenger RNA and its attachment of ribosome.

(4) Synthesis of virus coded early protein, viz. DNA polymerase.

(5) Replication of viral DNA using DNA polymerase and host cell nucleotides.

(6) Transcription of more messenger RNA molecules from newly formed viral DNA molecule. These are different from the ones synthesised previously (Step 3).

(7) Synthesis of coat proteins and liberating enzymes.

(8) Assembly of viral components into mature particles.

(9) Their release.

The important groups of DNA viruses whose process of replication have been studied fairly extensively include adenoviruses, herpes viruses and pox viruses. We shall briefly discuss here the process as operative in pox viruses (Joklik 1966).

Pox viruses are the largest and structurally the most complex of all viruses. A mature pox virus particle consists of a central nucleoid surrounded by bundles of protein bodies. There are two conspicuous lateral bulges near about the centre of these virus particles. They enter the host cell by the usual mechanism of phagocytosis. However, because of their complexity, the subsequent steps are long drawn and

complicated.

The mechanism of uncoating of the DNA starts after the virus is within the cell and is lying in a vacuole. First, the outer phospholipid and protein coat is removed. This is achieved by the activity of the

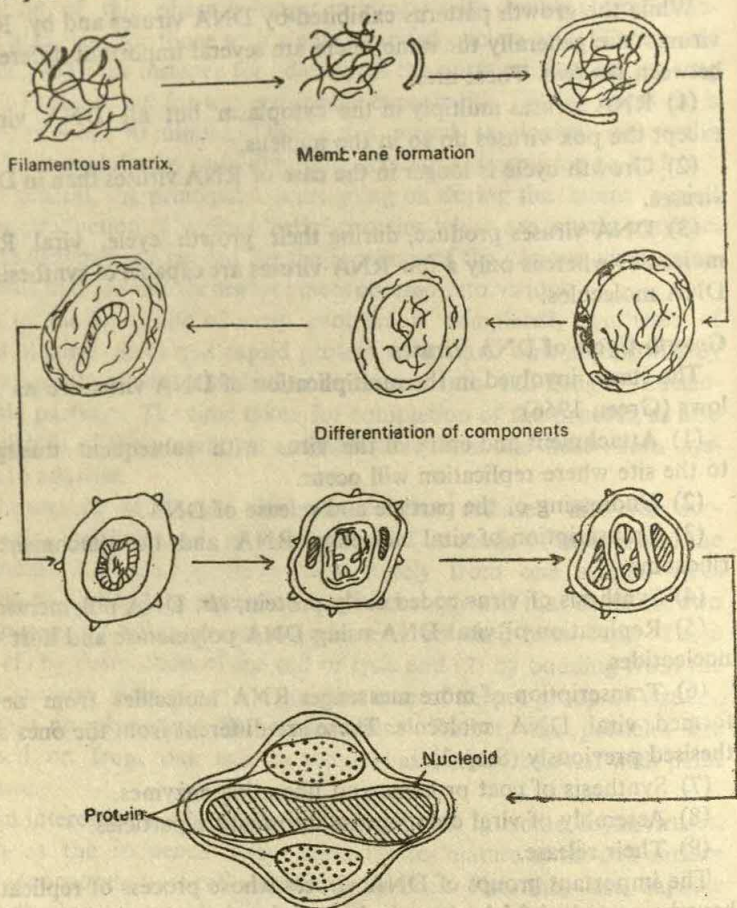


FIG. 5.11. Caccinia virus and its formation. These are examples of complex viruses. A mature virus consists of a central nucleoid surrounded by bundles of protein bodies. The most conspicuous characteristics of these particles is the presence of two lateral bulges near about the centre of these particle. The manner of its formation within the host cell is also interesting. A filamentous matrix is first formed. This is gradually enveloped by a membrane. The enveloped matrix is gradually developed into the nucleic acid core and the protein bundles. The membrane is also converted into a proper envelop. In this manner the mature virion is ultimately formed (after A.A. Avakhyan and A. F. Bychovsky).

enzymes of the host cell. Next there is a process of uncoating of the DNA by removing the remnants of protein layers. This step is mediated by enzymes synthesised by the partially uncovered viral DNA.

The released DNA may be found at one or at several sites in the host cell, depending upon the multiplicity of infection. As each site, also referred to as the *factory*, viral DNA synthesis starts. This is followed by synthesis of viral messenger RNA by the newly formed viral DNA. Subsequently several viral proteins are formed which ultimately constitute the coat.

While these steps are being carried out the individual factory sites are gradually covered by a membranous lipoprotein envelop. Maturation of the particle occurs inside these envelops. These may be retained by the cell or be released very slowly (Fig. 5.11).

Growth Cycle of RNA Viruses

The important RNA viruses whose growth cycles are somewhat clear include picornavirus, arboviruses, reoviruses and myxoviruses.

As with DNA viruses, the process of replication in RNA viruses also occurs in a stepwise fashion as follows:

- (1) Entry of the virus into the cell;
- (2) Undressing the virion to release RNA;
- (3) Synthesis of new viral RNA;
- (4) Synthesis of new protein;
- (5) Maturation *i.e.*, assembly to make new infective particles; and
- (6) Their release.

The process of replication in RNA viruses can be exemplified with the process of replication of fowl plague virus, a member of the myxoviruses which is closely related to influenza virus (Fig. 5.12).

Replication in fowl plague virus (FPV) has been studied in detail by W. Schafer and his associates. These particles contain what is called *g*-antigen (a mixture of viral nucleic acid, hemagglutinin and lipids) which helps both in attachment to and penetration of the host cell membrane. The viral particles enter the host cell and their nucleic acid is released. In the next phase the viral nucleic acid enters the cell and starts inducing the host cell nucleus for the synthesis of the viral components, the *g* antigen. These get localised at different sites in the host cell called the *factories*. Synthesis of coat protein units also begins. Subsequently, the *g*-antigen and protein units get assembled into virus particles. After assemblage, the particles get covered by lipid membranes. This membrane is, apparently, a residue of the host cell membrane. In other words, this component is not a result of some

active synthetic process. Rather, it provides an example of utilisation of the host cell component as such.

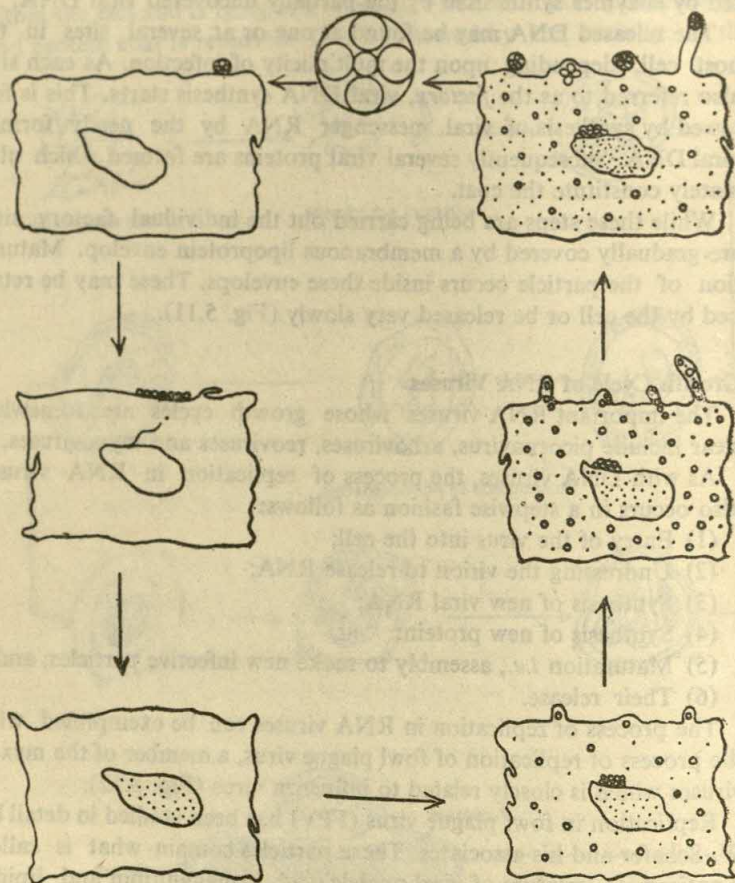


FIG. 5.12. The process of replication in fowl plague virus. These viruses are complex viruses made up of small sub-units (spherical diagram at the centre). The virus particles first get attached to the host cell surface and soon get separated into the smaller sub-units. These sub-units then enter the cell. The protein coat is removed and the nucleic acid induces the host cell nucleus to produce the 'g' antigen. Protein components are also synthesised. These incomplete viral components are then assembled into complete virus particles at certain specific sites or loci, called the *factories*, in the cytoplasm. These, then, come out of the cell, and restart the cycle.

ARTIFICIAL SYNTHESIS OF VIRUSES

Investigations on viral replication during the last two decades have clearly revealed that the virus nucleic acid, once within the host cell, directs the synthesis of its own protein coat out of the host cell metabolites. They also direct the different host cell enzymes for the purpose. Once some information regarding the various aspects of this phenomenon was obtained, scientists started thinking in terms of experiments designed to simulate the sequential natural events under laboratory conditions. The apparent simplicity of the process makes one hopeful about the outcome of these attempts which in fact are steps towards creation of life itself.

The first problem in this connection was the creation of suitable preparations of viral nucleic acid. Once a new molecule is synthesised *in vitro* using appropriate precursors, it could be used for guiding the construction of viral proteins. One such step would be to use a natural template for the synthesis of the new DNA molecules. Obviously the template molecule must be isolated from a natural virus particle. An artificially created molecule can thus be obtained by using such a template. Other steps like synthesis of messenger RNA molecules and various enzyme proteins, could follow subsequently.

Some success in this regard was achieved in 1967 with the bacteriophage ϕ X174 by Arthur Kornberg and his associates at Stanford University and by R.L. Sinsheimer at California Institute of Technology, all working as a team. You will remember this virus as the one with single stranded DNA of circular appearance. When this virus infects its host, its nucleic acid strand acts as a template and synthesises a complementary strand known as the replicative form. Both the strands are infective. The original strand is known as the (+) type and the newly synthesised one as the (-) type. Needless to emphasise that if the new strain is not a perfect replica of the original one, the replicative form, even if formed, remains non-infective.

Kornberg and his associates started by designing experiments with a view to synthesising new DNA strands utilising ϕ X174 nucleic acid as the template. The enzyme used for the purpose was DNA polymerase from the bacterium *E. coli*. This enzyme had earlier been obtained in a highly purified form by these scientists and was shown to be capable of *in vitro* polymerisation of precursor nucleotide in the presence of purified DNA molecules acting as template. Earlier, although it had been possible to obtain physically detectable new molecules, their biological purity could not be ascertained. ϕ X174 DNA, if properly replicated could provide an answer to the problem.

For, if the new strand showed infectivity, the replicative form had to be an exact replica of the original one.

Earlier experiments proved unsuccessful in the sense that the molecules did not possess infectivity. The reason for this was soon found out as it was discovered that the new molecules were linear in arrangement and not circular as in the case with naturally obtained DNA molecules of this virus. Obviously, the circular form was an important factor to be considered. It was also found out that a different enzyme was needed to join the two ends of the linear molecule and convert it into the circular form. This enzyme is known as ligase or the joining enzyme. A purified preparation of this enzyme was obtained and used for the formation of a circular strand. At last success was achieved. A new strand could be artificially synthesised in an infective form. And since it was infective, it was obviously circular.

Such artificially prepared strands could be separated from their mother strands by density gradient ultra centrifugation. By this method molecules of different masses could be segregated into separate bodies even when they are in solution. Therefore, separation of new molecules would be feasible only when these are heavier or lighter than the original ones. The best way to synthesise heavier strands would be to incorporate heavier precursor molecules. This can be done by using nucleotides with modified nitrogenous base that are heavier than the normal ones. One heavy base commonly used is bromothymine, a derivative of thymine, the important base present universally in DNA molecules.

After the separation of newly synthesised nucleic acid strands are thus achieved, the next step would be to use these new strands for the synthesis of specific protein coats. The last objective has not been realised fully till now. The difficulties encountered are many. For one thing, even if it were possible to synthesise a few polypeptides using the newly synthesised DNA as template, it would be very difficult to achieve the synthesis of exact protein monomers having specific properties and enzymic activities. These objectives are yet to be realised.

Another important aspect of these experiments is that in each case no new sequence or code is created afresh. The template used is natural and hence the sequence of nucleotides used had already been there. In order to synthesise a new virus in its entirety from the various raw materials, it would be imperative that a new DNA be synthesised. Although it has been possible to synthesise polymers of several nucleotide length (equivalent to small fragments of nucleic acids) by Professor Har Gobind Khorana and his associates at the University of

Wisconsin in the USA, the real objective is still a far cry.

For one thing, to synthesise a complete viral nucleic acid molecule, the sequence of nucleotides making it up must be known first. Only recently the sequence of DNA of one of the small viruses, Coliphage ϕ X174, has been fully elucidated (Sanger *et al.* 1977). This is the first report of complete nucleotide sequence of any virus. Artificially synthesising even this known and comparatively small sequence of nucleotides (5375 in all) is considered to be a stupendous undertaking. However, attempts in this regard are being made and success is distinctly considered to be within the realm of possibility. Synthesis of proteins utilising this artificially created nucleic acid would form the next step. This would be followed by the proper assembly of the nucleic acid molecules and the protein subunits into mature nucleocapsid particles. Only when such artificially created nucleocapsid particles are infective it can be said that artificial synthesis of viruses has been achieved.

GENETICS OF VIRUSES

The viral genome, whether DNA (deoxy ribonucleic acid) or RNA (ribonucleic acid) is the sole repository of all genetic characteristics of a virus species. As do the genomes of all living organisms, the viral genome too codes for the functional and structural proteins in a precise, sequential and well regulated manner, ultimately culminating in the production of a large number of virus particles of the same genetic type. That there is a necessity of a specific host cell at the physiological level to carry out all these functions does not in any way diminish the importance of the genetical control exercised by the virus genome over its entire range of functions; it merely makes it unique.

Indeed, it would not be out of the mark to say that the style of functioning of the virus genome is quite ruthless in as much as it makes the host genome totally subservient to itself. Even the formidable immune response system of the vertebrates sometimes fails to contain the viral onslaught. The existence of latent, and possibly forcible, association between the host and the viral genome also serves as an indicator to the adaptable and stable nature of the viral genome.

In the previous chapters we have seen, in a general way, how the viral genome functions at the physiological level. In the present, we propose to look into some aspects of the current understanding regarding the nature of the viral genome and into the genetical phenomena associated with the viral life process.

Early Studies

The bacteriophage-bacterium system, because of the overall convenience it provided, had developed during the early forties, into a widely utilised system for studying the process of bacteriophage replication. The typical one step growth curve was established by Prof. Max Delbrück and his associates during this period. Formation of well defined and characteristics plaques by bacteriophages on bacterial 'lawns' was well recognised.

In 1946, Prof. A.D. Hershey described a series of naturally occurring mutants of the coliphage T_4 . Further, he found some of these mutants

to be *defective* meaning that they had lost the capacity to lyse certain bacterial strains and form plaques. Naturally occurring mutants of tobacco mosaic virus (TMV) were also recognised (Jensen, 1937) and difference in their protein amino acids demonstrated (Knight 1947).

At about the same time, several interesting phenomena relating to simultaneous infection of a host bacterium by related and unrelated bacteriophages were reported by Delbrück and associates (Delbrück and Bailey 1946). For instance, it was found that mixed infections often led to interference of the replication of one virus by the other. The phenomenon of self-interference *i.e.*, prevention of infection by one particle in the presence of another particle of the same type, was noted. Occurrence of stable new phage types as a result of mixed infections was observed. It was attributed at that time to spontaneous occurrence of mutations (Delbrück and Bailey 1947). However, later events led one to conclude that some of these at least were the result of recombination.

In the early fifties, brilliant experimentation by Hershey and Chase (1952) conclusively proved that viral DNA was the genetic principle responsible for successful infection and replication. Biological role of DNA as the genetic material in bacteria was reported earlier by Avery and his associates (Avery *et al.* 1944). The implications of the new discovery were quite clear. Genetical changes noted hitherto in viruses could now certainly be attributed to the genetic material *i.e.*, DNA, itself.

A few years later it was reported with certainty that viral genomes could recombine as well. For example, a T_2 coliphage mutant forming small dark plaques and another forming large light ones, on simultaneous infection, gave rise to large dark and small light ones besides the parental types (Benzer 1955). In another experiment two bacteriophage mutants *r IIA* and *r IIB* were used. Both of them were capable of infecting *E. coli* strain *B* but not *E. coli* strain K_{12} (λ), which could be infected by wild types of the phage only. However simultaneous infection of the strain *B* by both the phage mutants resulted in formation of a few recombinants (one in 10^6 cells) capable of infecting the strain K_{12} (λ) as well. This was obviously a case of recombination.

A number of characters were shown to be capable of undergoing recombination under controlled laboratory conditions. Analyses of such recombination data indicated that these characters were in a linear order as on a chromosome (*see* Benzer 1955; Hershey 1957).

Another remarkable discovery to be made during the early fifties was that there could be an interchange of heritable characters between a bacteriophage and its host bacterium. Such interchange was spon-

taneous and led to the discovery of phage mediated transformation (transduction) in bacteria (Zinder and Lederberg 1952).

The other observation of significance was the ability of certain phage genomes to be intimately associated with the genome of the host cell. Bacteriophages in such a state lost their ability to kill and lyse the host cell and became *temperate* (Lwoff 1953). This process, named *Lysogenisation* was reported to induce permanent hereditary change in the host.

All these genetical phenomena are reported to occur spontaneously in nature. However, these have been re-enacted under controlled laboratory conditions as well. Different host-virion systems have been and are being used by researchers all over the world to elucidate the various aspects of these phenomena. In the following paragraphs our attempt would be to summarise the current understanding regarding these phenomena as understood in the light of current researches.

MUTATIONS

Mutations are defined as alterations in the genetic material leading to permanent hereditary changes in the organism concerned. Mutations, both natural and induced, are known to occur in the virus genome. Indeed, much of our current understanding of molecular biology can be traced to viral, particularly bacteriophage mutations.

In viruses, mutations, as expressed phenotypically, mainly relate to their behaviour towards their respective hosts. However, mutations relating to their ability to grow only under certain conditions or to changes in the morphology of the particles are also known to occur.

Mutation in Bacterial Viruses

Bacteriophages show two principal types of mutants. These are:

- (a) Plaque mutants; and
- (b) Conditionally lethal mutants.

Plaque mutants. The plaque mutants are normally expressed as variations in the morphology of the plaques produced by a phage on the *lawn* of its host bacterium. These variations appear because of

- (i) change in the host range; and
- (ii) ability of mutants to lyse the host cells at rates different from that of the wild type.

It has been known earlier that plaques formed by genetically distinct phages are easily distinguishable. For example, the plaques of coliphage T_2 could easily be segregated from those made by coliphage λ . Similarly many wild types T_2 and T_4 phages are known to

produce plaques morphologically different from their wild types.

Secondly, such changes could arise due to difference in the rates of lysis of the host cells. These mutants, called the rapid lysis (*r*) mutants are particularly observed in *T* even phages. For example, the rates of lysis of the host cell by T_{2r} (wild type) is much slower than the rate of its mutant T_{2r} (Plate X). Rapid lysis would lead to formation of larger plaques in even time.

Another type of mutation could lead to change in host range because of the ability of the mutant to adsorb on a new host. For instance wild type T_2 cannot multiply on *E. coli* strain *B/2* because it cannot adsorb on the bacterium. Mutant T_2 particles can, however, adsorb and multiply on *B/2*. This ability is gained because of altered tail fibers.

Conditionally lethal mutants. Conditionally lethal mutants are developed because of sudden requirement of some specific necessity. For example, certain coliphages which have the ability to multiply at 25°C but cannot multiply at 42°C. These are called temperature sensitive conditional lethals. This inability could be due to destruction of the three dimensional structure of a protein necessary for their reproduction.

Under certain conditions, the acquired lethality may be complete. For example, the mutants of lysogenic phage (λ) which fail to be induced into the lytic form. This could be due to the failure of the prophage (on induction) to synthesize the requisite nucleic acid molecules and the protein monomers. Sometimes, even partial ability to produce the essential macromolecular components may lead to the production of defective phages. As for example certain mutants of phage λ and of simian virus 40.

Mutations in Animal Viruses

Existence of different pathogenic strains of animal viruses, such as influenza virus, was known. However, one of the first reports of isolation of different strains of influenza virus from infected human patients was that of Prof. McFarlane Burnet and his associates (see Burnet and Bull 1943). These strains differed in their virulence and rapidity of growth. At present a number of mutants, both natural and induced, of such well recognised virus groups as polioviruses, influenza virus and poxviruses are known. (see table 6.1).

In recent years a large number of mutant types belonging to these group of viruses and to some others have been recognised. The criteria of recognition have been (i) infectivity and host range, (ii) haemagglutination, (iii) neuraminidase synthesis (in case of influenza virus), (iv) temperature sensitivity (polio virus), (v) antigenic properties and

(vi) nature of plaque or pock.

While much work has still to be done to elucidate the nature of these mutations, it is increasingly becoming clear that the changes in the protein coat and its components are basically responsible for the expression of the various mutations.

TABLE 6.1. MUTANT TYPES ASSOCIATED WITH
ANIMAL AND PLANT VIRUSES

| <i>Mutant Type</i> | <i>Characteristics</i> |
|------------------------------|---|
| Morphological | Typical lytic symptoms on the culture plate |
| (a) Plaque type | |
| (b) Pock type | |
| Surface mutant | Physical changes in the viral morphology |
| Antigenic mutants | Changes in the antigenic properties of the virus |
| Pathogenic mutants | Changes in the capacity to infect pathogenically |
| Host range mutants | Alterations in the host range |
| Resistant mutants | Types with acquired resistance against antiviral agents |
| Conditionally lethal Mutants | Do not survive under changed cultural conditions. |

Mutation in Plant Viruses

Several well studied plant viruses, such as Tobacco Mosaic Virus (TMV) and Tobacco Necrosis Virus (TNV) are reported to have a wide range of spontaneously occurring mutants. Jensen (1937) was able to distinguish between three spontaneously occurring TMV mutants on the basis of disease symptoms and infectivity on different strains of tobacco. Since then a large number of mutations have been recorded in TMV. These mutations have mostly been induced by such mutagenic agents as nitrous acid and ultra-violet radiation.

Plant virus mutants are generally recognised by changes in (1) host specificity; (2) disease symptoms; (3) serological specificity (antigenic properties); (4) virulence or pathogenicity; and (5) protein structure. Sometimes, the total loss in capacity to infect also acts as a criterion as for example in the PM series of TMV mutants (Siegel 1965).

All these types of mutants have been shown to be differing in both physical and chemical properties. Thus, change in antigenic properties, amino acid composition, position and sequence of individual amino acids, and the nature of aggregation of polypeptide chains into pro-

tein monomers have all been shown to be occurring (see Smith 1974, Knight 1974).

Frequency of Mutation

The frequency of spontaneous mutation in viruses has been ascertained in a number of cases. The frequency with which different marker traits are recognized as to have mutated falls within a considerable range, even in case of the same virus. For instance, it has been noted that spontaneous mutation of coliphage T_2 may produce plaque type mutants at the frequency of 10^{-4} per duplication (*i.e.* plates). However, it may produce host range mutants only at a frequency of 10^{-8} per duplication. A difference of the order of 10^4 (Stent 1971)!

A similar range of frequency has been calculated for RNA and DNA containing animal viruses. The frequency of mutation in plant viruses is not well known. However, according to one calculation it may be as high as 0.5 per cent (Kunkel 1940).

MECHANISM OF MUTATION

Mutations, being heritable changes, have been explained in terms of the central dogma of molecular biology:

DNA \rightarrow mRNA \rightarrow SRNA \rightarrow protein.

Accordingly, any heritable character can be altered only when DNA *i.e.*, its nucleotide sequence gets changed. Or, alternatively it starts permitting special type of function under special conditions. In both cases, considerable amount of flexibility is attributed to the structure and function of DNA. This role would hold true for virus genome as well. Additionally, such a role has to be envisaged for RNA which is the sole genetic material in a large number of viruses.

Change in polynucleotide sequence can be brought about by substitution, alteration and deletion of any of the component nucleotides, at one or more sites. Such a change should completely alter the reading frame of the sequence, partially or even totally. This new reading frame would be reflected in the synthesis of an altered polypeptide or protein monomer. In case of viruses this alteration will be registered in a large number of characters whose expression is dependent upon the protein coat.

In an interesting experiment it was shown by Rogers and Pfuderer (1968) that the genome of a TMV could be altered artificially by treating it with adenosine diphosphate (ADP). The genome (RNA) of the virus was incubated with ADP and RNA polymerase. A RNA molecule was obtained which had 18 additional adenine nucleotides.

As AAA codes for lysine it was supposed that the protein synthesized by the virus should now contain extra lysine residues. The altered RNA molecule was injected into healthy tobacco plants. New particles were formed. Their protein coats did not show much change but additional peptides containing only lysine were isolated. In other words, the altered RNA did function normally. Only the new peptide synthesized by it was not needed for coat formation.

GENETIC RECOMBINATION IN VIRUSES

In 1946 Dr A.D. Hershey described a variety of spontaneously occurring mutations in *T*-even coliphages. One class of these mutations were shown to affect the type of plaques. The most conspicuous of these mutants are the *r* mutants. These mutants differed in their rapidity to lyse *E. coli* cell. Some of these *r* mutants of coliphage T_2 and T_4 , like T_{2r} and T_{4r} differed from the wild types, $T_{2r}+$ and $T_{4r}+$ respectively, in that they could not infect and lyse *E. coli* strain *B* whereas the wild type could. However, the mutants could be grown on other *E. coli* strains like K_{12} (λ) or B_4 .

At about the same time Max Delbrück and his associates were studying the phenomenon of mixed infection of *E. coli* by different pairs of phage mutants. In one such experiment they used two mutants, namely, the strains $T_{2r}+$ and T_{4r} . First they allowed them to simultaneously infect a common bacterial strain. Then they plated these infected bacteria on the same or another bacterial strain. The originally infected bacteria got lysed and liberated progeny particles. The progeny particles, in their turn infected cells of the indicator bacteria and formed plaques.

In one such experiment, the primarily infected bacterium was allowed to infect cells of *E. coli* strain B_2 used as the indicator. Normally since neither $T_{2r}+$ and T_{4r} were capable of infecting B_2 , no plaques should have been formed on it. However, they found that some mottled plaques were found even on B_2 indicating that some new types having wild type properties were created during the mixed infections. Delbrück and Bailey explained the phenomenon by suggesting that there had been a mutation at the *r* locus of T_{4r} type converting it to $T_{4r}+$ type which was capable of infecting and lysing *E. coli* strain B_2 . However, they did not rule out the possibility of any other explanation and even suggested that in some respects there were more line 'transfers or even exchanges, of genetic materials.'

A few years later, it was shown that indeed such changes are due to transfer or exchange of the genetic material. Working with *rII* mutants

of coliphage T_4 , Hershey and associates had shown that recombinants appear capable of behaving like wild types. The rII mutants are characterised by their ability to infect *E. coli* strain B and their inability to produce plaques on *E. coli* $K12$ (λ). Different rII mutants like $rIIA$ and $rIIB$ differ in their rates of lysing the same bacterial strain.

Two rII mutants, A and B were made to simultaneously infect *E. coli* strain B . The progeny phage particles from infected bacteria were then plated separately on cultures of *E. coli* B and *E. coli* $K12$ (λ). The composition of the progeny particles was found to be interesting. While some of them showed the characteristic plaques of the parental types on strain B , some others were capable of forming plaques even on *E. coli* $K12$ (λ). Since both the rII mutants were incapable of forming plaques on $K12$ (λ), new types or recombinants were obviously formed (Hershey and Rotman 1949).

Similar but detailed studies by Seymour Benzer enabled him to genetically map the r locus and to elucidate its detailed or fine structure (Benzer (1955)). In one experiment two mutants of Coliphage T_2 namely T_2hr and $T_2h^+r^+$ were used. These mutants are double mutants one at the locus h and other at the locus r . T_2hr can grow on both *E. coli* strains B and $B/4$, where as the strain $T_2h^+r^+$ can grow only on *E. coli* strain B . Further, T_2hr forms larger and clearer plaques, whereas $T_2h^+r^+$ forms small wild type plaques. $T_2h^+r^+$ strain is also referred to as the wild type respect of these two genes. When these two mutants were made to infect susceptible *E. coli* B cells simultaneously, four types of progeny particles are found. These were T_2hr , $T_2h^+r^+$, T_2hr^+ and T_2h^+r . These could be easily detected by plating the progeny particles in a mixed culture of *E. coli* strains B and $B/4$. In such a mixed culture, the viral phenotypes would be manifested as follows:

$T_2h^+r^+$ = Small turbid plaques

T_2hr = Large clear plaques

T_2h^+r = Small clear plaques

T_2hr^+ = Large turbid plaques

Turbid plaques would appear because the mutant phage concerned (T_2hr^+ or T_2h^+r) will not be able to kill $B/4$ cells but would kill B cells. Since $B/4$ cells would not be killed, the plaques formed would be partially clear and assume turbidity (See plate XI).

The recombinants formed in these cases often went upto 50 per cent. Recombination values for other gene locuses were also determined and used for genic mapping of T even phages (See Benzer 1955, 1957).

Mechanism

A very significant aspect of mechanism viral genome recombination is that, unlike in other true living organism, here the entire process is not restricted to a pair of genomes. Rather, it occurs amongst all the newly synthesised DNA molecules present in the host cell. These molecules are the direct products of the two infecting viral genomes and, therefore, represent two different genomes. While these molecules are being formed by successive divisions, mating between two different molecules might easily occur. Such mating could be partial or total and generally involves the strand which is being newly synthesised. A fragment of this chain could be preferentially exchanged with a similar fragment of another. This mechanism has been referred to as copy-choice (Fig 6.1).

This type of mating might occur between molecules of the same generation, between molecules of different generations and between already recombined molecules. The process, therefore, is more complex than would otherwise appear to be. As a result of such mating, some unique phenomena like heterozygous DNA and assembly of different segments in the same region of the DNA are known to occur.

Apart from bacterial viruses, animal, insect and plant viruses are also known to express manifestations of genetic recombination (see Waterson 1968). For example, it has been noted that when two non-oncogenic (non-tumour producing) viruses were made to infect a particular host at the same time, the host developed tumorous growths after a lapse of time. It was concluded that this was due to a recombined viral DNA that caused the new expression. Genetic recombination in RNA viruses is also known. With influenza viruses, particles are reported to become infectious when the host is infected by two or more ineffective viral strains.

Frequency of recombinants in such cases is, however, smaller than that observed in case of the DNA viruses. Antigenic variability of these particles are also known to increase due to recombinations. Mixed particles are known to form as a result of recombination between two particles during that developmental stages *e.g.*, in pox viruses. The particles of these agents are assembled in the so called factories in the host cell, where the chances of recombination between the two genomes are fair.

The phenomenon of recombination is of considerable significance. Biparental (between two parents) or triparental (involving a fresh recombinant and a third genome) crosses result into progeny equipped with genetic components of more than one or even two genomes.

Consequently, there is an increase in the genetic potentialities of the newly formed particles. As it has been shown, ineffective strains of

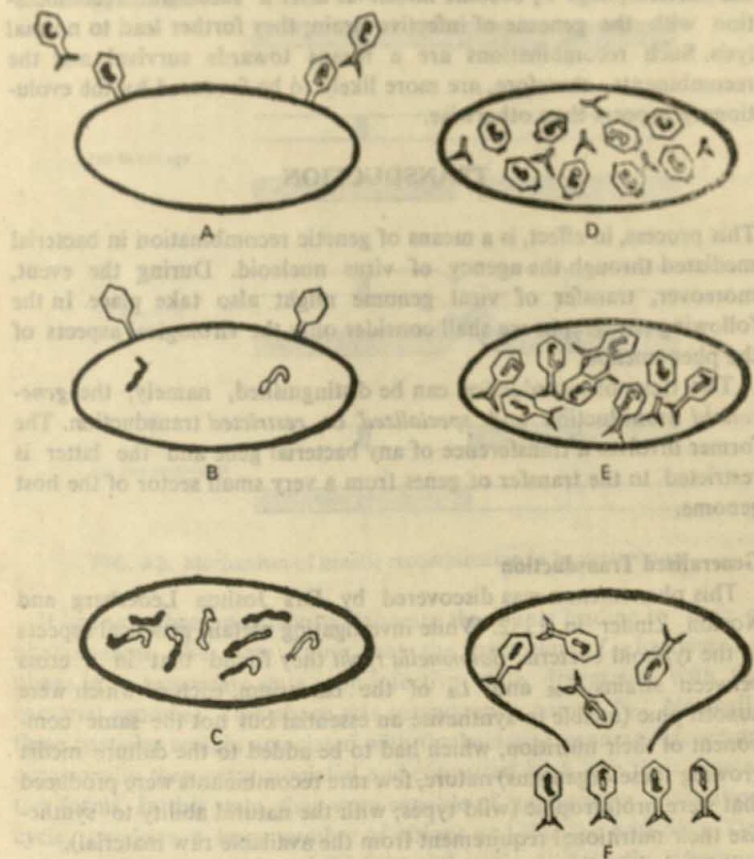


FIG. 6.1. Genetic recombination in bacteriophages. Sometimes two slightly dissimilar phages infect the same host bacterium at the same time. When the process of replication is complete and the host cells are lysed, the resultant progeny of these two phages are observed to be of four types; two of them are found to be of the parental nature and the other two, recombinants. Such recombination is rare. And the recombinants are genetically stable. Such a phenomenon occurs due to recombination amongst the nucleic acid molecules of the two parental types. This happens during replication of the phage nucleic acids and is analogous to the process of recombination in eucaryotic organisms. The mechanism is known as 'copy choice.'

The diagram here shows the formation of recombinants having two different types of tail fibres.

the bacteriophage T_2 become infectious after a successful recombination with the genome of infective strain; they further lead to normal lysis. Such recombinations are a means towards survival and the recombinants, therefore, are more likely to be favoured by the evolutionary process than otherwise.

TRANSDUCTION

This process, in effect, is a means of genetic recombination in bacterial mediated through the agency of virus nucleoid. During the event, moreover, transfer of viral genome might also take place. In the following paragraphs we shall consider only the virological aspects of the phenomenon.

Two types of transduction can be distinguished, namely, the *generalised* transduction and *specialized* or *restricted* transduction. The former involves a transference of any bacterial gene and the latter is restricted to the transfer of genes from a very small sector of the host genome.

Generalised Transduction

This phenomenon was discovered by Drs Joshua Lederberg and Norton Zinder in 1952. While investigating certain genetical aspects of the typhoid bacteria, *Salmonella typhi* they found that in a cross between strains L_{22} and L_2 of the bacterium, each of which were auxotrophic (unable to synthesise an essential but not the same component of their nutrition, which had to be added to the culture media growing these organisms) nature, few rare recombinants were produced that were prototrophic (wild types; with the natural ability to synthesise their nutritional requirement from the available raw material).

It was originally supposed that this change was due to bacterial conjugation (direct incorporation of the genome of one bacterium into another). However, when the same experiment was repeated by growing the strain L_{22} in bacteria free mixed culture filtrate of the strains L_{22} and L_2 , similar conversion was observed to occur in the same frequency. Obviously some entity in the nature of filtrable agents was present in the bacteria free culture filtrate, which might be responsible for the change. One hypothesis was that this was a fragment of the bacterial genome which managed to pass through the bacteria retaining filters. However, it was shown that this agent was resistant to DNase and therefore could not be a fragment of bacterial DNA. It was proved by them subsequently that this agent was in fact a virus—a phage (Fig. 6.2).

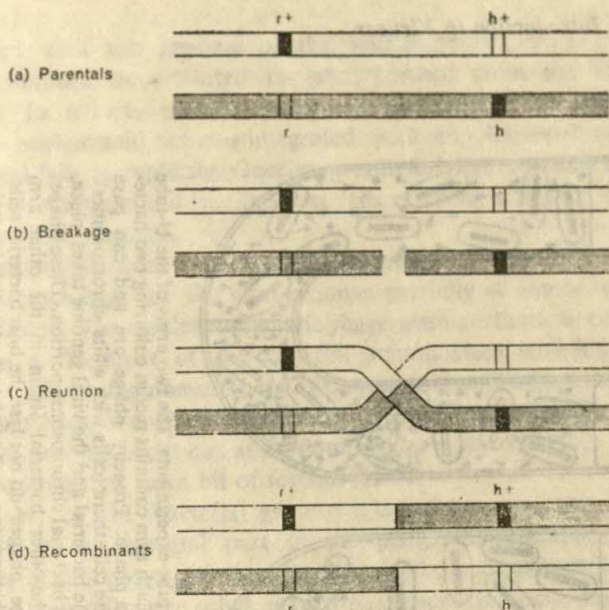


FIG. 6.2. Mechanism of genetic recombination in bacteriophages.

It has since been possible to elaborate the events leading to such a phenomenon. We now know that the bacterium L_{22} harboured a phage in its temperate state (non-infective state, integrated with the bacterial genome). This phage was subsequently named P_{22} . Normally these particles remain associated with the bacterial genome. However, occasionally they get dissociated and converted back into their vegetative forms. In this state, they were capable of undergoing replication cycle, produce a large number of virions and lyse the host cells. The progeny of P_{22} then infected the bacterial strain L_2 . Initially, it became temperate in L_2 . Later, after many generations it became dissociated and the normal replication cycle followed, leading to lysis of the host cell. The released progeny virions then reinfected the bacterial strain L_{22} but, instead of multiplying and causing lysis, the virions once again became temperate. As a result of all this, the auxotrophic L_{22} strains were converted into prototrophs. Since such a transfer was hereditary, transfer of gene was obviously involved.

Mechanism of Transduction

The mechanism of transduction is elegant and simple (Fig. 6.3). The nucleic acid of a phage in its temperate state normally remains

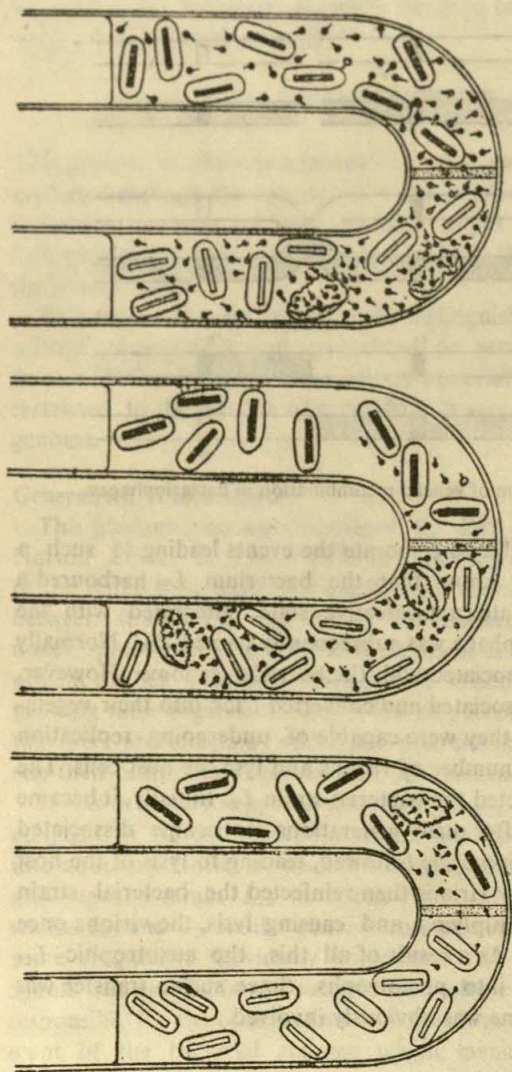


FIG 6 3. Diagram depicting the process of transduction in U-tube experiments. The two arms of the U-tube are separated by a sintered glass bacterial filter. Each arm contains media culturing two bacterial strains. One of the cells in the right hand arm is infected by a phage. Progeny phage are and can pass through the filter into the other arm, and rarely, infect one of the cells there (cells with white chromosomes). The phages multiply and during the process exchange between the bacterial and the viral genome takes place. As a result, a few of the newly formed phages come to have the bacterial chromosome portion. These phages then again pass through the glass filter and reinfect the cells of the other bacterial strain in the other arm (cells with black chromosomes). However, on infection these phages do not lyse the host bacterial cells. Rather, the phage nucleic acid gets integrated with the host cell chromosomes. The phages with the bacterial chromosome fraction are also similarly integrated. Therefore, the corresponding genes of the first bacterium are transferred to the second one (black chromosome with white area). And along with it, the characters are also transferred. Such a phenomenon is extremely rare and occur in the frequency of 10^{-7} .

integrated with the genome of the host bacterium. Occasionally it may be induced or activated to get separated from the bacterial genome. In nature, such a separation is unusual and rare. Normally the viral nucleic acid remains integrated with the bacterial chromosome and fails to replicate. Once segregated, however, these become vegetative, multiply and lyse the host cells releasing a large number of progeny virus particles.

The most significant event during this process of segregation is an intricate exchange of the viral genome, partially of course, with the bacterial one. The resultant bacteriophage genome thereon continues to have a fragment of the bacterial genome along with it and thus becomes a hybrid genome. These are later on assembled into bacteriophage particles. We do not know how an exactly matched size of the bacterial genome is cut out at random and how the assembly occurs, incorporating that extra bit of foreign DNA.

This bit of the bacterial genome is called the *exogenote*. It starts behaving as an integral part of the phage genome. The progeny particles of this phage genome also contain the same exogenote. These particles then infect the other auxotrophic bacterial strain and again become temperate. In other words, the phage nucleic acid carrying the exogenote fails to replicate and becomes integrated with the bacterial chromosome of the new host. The new bacterial genome, also called the *endogenote* thus receives the exogenote. The recombinant genome is hybrid in nature and is called the *heterogenote* and shows prototrophic traits.

Generally one, and rarely closely linked, genes are transduced. The size of the bacterial DNA segment, usually about 1 to 2 per cent of the bacterial genome, should not only be small but also be capable of being accommodated, along with the viral DNA, inside the phage head.

In addition to *Salmonella*, transduction has been reported with different phages for such bacteria as *Escherichia coli*, *Shigella*, *Pseudomonas*, *Bacillus*, *Staphylococcus* and *Proteus*. Usually a high titre phage suspension is used while performing transduction experiments in laboratories. The frequency of transduction of a given character ranges from 10^{-8} to 10^{-5} per cell.

Specialized Transduction

As mentioned earlier, this category of transduction is restricted in the sense that transfer of a very small region of the host chromosome is involved. For a particular strain, this region is usually specific. It was demonstrated by Dr Joshua Lederberg and his associates that the

strain K_{12} (λ) of the bacterium *E. coli*, auxotrophic for galactose was transduced by the phage lambda only for a small segment of the genome responsible for the synthesis of galactose.

It was shown by them that prototrophic host cells carrying the gene for galactose synthesis (*lac* gene) also harbour the integrated prophage or the viral nucleic acid in its temperate state. In rare cases, the integrated prophages are induced into their virulent state and consequently lyse the host bacterium. This results into the release of a large number of infective particles in the lysate. When the lysate comes in contact with developing cultures of auxotrophic strains, the latter get transduced. It was explained that the free form of the phage in the prototrophic cell carried along with it the gene responsible for galactose synthesis. Another significant observation was that sometimes the prototrophic bacterium having the gene for galactose synthesis is not lysed. Nevertheless, when the auxotrophic strains come into contact with the culture filtrate of the protroph, transduction still results.

This category of transduction is different from the type described earlier on several counts. The most significant of these is that this type does not necessitate lytic infection of the bacteria carrying phage genome, the exogenote. Induction leads to the conversion of the temperate phage into the free form which often is released into the lysate. When the phage sensitive auxotrophic bacterial strain is infected with the lysate, the phage gets incorporated into the endogenote. Other differences include the fact that the exogenote is located on the bacterial chromosome near the point of infection by the prophage. The third point is the comparative instability of this type of transduction (Fig. 6.4).

Transduction Involving Other Host-Virion System

It is very difficult to say whether transduction is a natural phenomenon in other host-virion systems too. However, instances where virions in the temperate state get naturally converted into the lytic state and destroy the host cells, are there; sudden appearance of such diseases as small-pox and encephalitis without any apparent source of infection, is an example of such behaviour. The inability to detect transduction stems from the main reason that, apart from a few well known examples like the bacteria, the chromosomes of various organisms are not properly mapped. Therefore, it is almost impossible to detect the addition of a gene at random unless it is a specifically identified one.

Some recent reports claim the occurrence of the phenomenon in

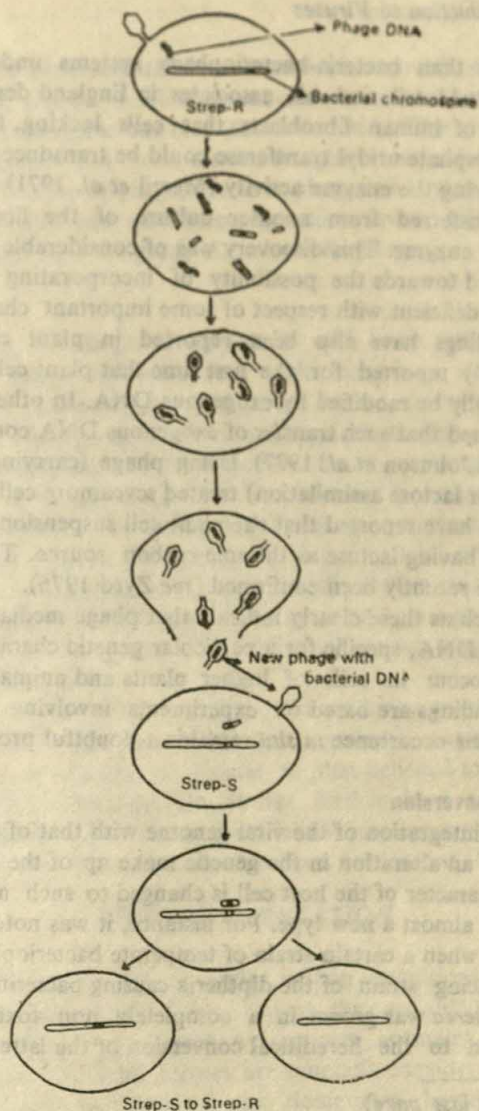


FIG. 6.4. Generalised transduction. An infective virion first infects a susceptible let unsay, Streptomycin resistant bacterial cell. The bacterial genome soon gets disintegrated. New phage nucleic acid molecules are also synthesised. When assembly of these nucleic acid molecules into new phage particles takes

(Contd. on next page)

systems other than bacteria-bacteriophage systems under artificial conditions. Dr Merrill and his associates in England demonstrated, using cultures of human fibroblasts that cells lacking the enzyme salactose-1-phosphate uridyl transferase could be transduced by phage into cells showing the enzyme activity (Merrill *et al.* 1971). The exogene was transferred from another culture of the fibroblast cells possessing the enzyme. This discovery was of considerable significance since it pointed towards the possibility of incorporating new genes into genomes deficient with respect of some important characteristics.

Similar findings have also been reported in plant cell systems. Ledoux (1974) reported for the first time that plant cells in culture could genetically be modified by exogenous DNA. In other reports it has been claimed that such transfer of exogenous DNA could be phage mediated (see Johnson *et al.* 1977). Using phage (carrying *lac* genes, responsible for lactose assimilation) treated scycamore cell suspension, these workers have reported that the plant cell suspension could grow on a medium having lactose as the sole carbon source. This observation has more recently been confirmed (see Zyrd 1976).

Reports such as these clearly indicate that phage mediated transfer of exogenous DNA, specific for a particular genetic characteristic, can conceivably occur in cells of higher plants and animals. However, since these findings are based on experiments involving cells grown in culture, their occurrence *in situ* remains a doubtful proposition.

Lysogenic Conversion

Lysogenic integration of the viral genome with that of the host cell results into an alteration in the genetic make up of the latter. Sometimes, the character of the host cell is changed to such an extent as to make it almost a new type. For instance, it was noted as early as in 1951 that when a certain strain of temperate bacteriophage from a toxin producing strain of the diphtheria causing bacterium *Corynebacterium diphtheria* was grown in a completely non toxin producing strain, it led to the heredital conversion of the latter into a toxic

(Contd. from last page)

place, an exchange between the bacterial chromosome fractions and the phage DNA may rarely take place. New phage particles thus possess bacterial DNA fragments. When these infect another bacterium which is streptomycetes, sometimes a very extraordinary thing occurs. Despite infection, the bacterial cells do not lyse. The phage genome carrying the bacterial genome fragments becomes integrated with the bacterial genome. In other words, a few genes of the first bacterium now get integrated with the genome of second one. The characters are also simultaneously transferred.

producing one. Since diphtheria is caused as a result of the toxin secretion the change in effect meant conversion of a non-pathogenic strain into a pathogenic one (see Zabriskie 1966). This type of hereditary conversion has been termed *lysogenic conversion* or *infective heredity*. In the case of phage, the term phage conversion is also used. Phage conversion has also been reported in several other groups of bacteriophages, e.g., the group *E. Salmonella* phages.

Mechanism

Mechanism of phage conversion is still to be fully worked out. There is no doubt, however, that such conversion is linked with lysogenisation (association of a phage in a temperate state) of the bacterial cell undergoing conversion. Therefore, the new traits come to be displayed because of integration of new genetic material in the form of the prophage. But it has been generally accepted that the prophage genome remains repressed while it is temperate and that is why it cannot replicate independently. Expression of certain genes of the prophage in the form of new traits of the host bacterium would, therefore, mean that while a part of the prophage remains repressed, another part remains active. The former are termed *lysogenes* and the latter *conversion genes*.

It has been suggested recently that conversion genes are portions of the bacterial genome which got segregated sometimes during evolution in a mechanism similar to that believed to be occurring during specialized transduction. It has further been suggested that their integration occurs on the bacterial chromosomes near the region where bacterial genes for similar functions are located (Uetake 1976).

COMPLEMENTATION

Some mutant virus strains under certain conditions, may remain functionally incomplete. Thus, though they are able to infect, some of their hosts strains are unable to replicate normally and in some cases not at all. Such viruses are generally considered as defective with respect to their specific hosts. Sometimes, however, they can replicate in the presence of a second virus which acts as a *helper* virus. The *helper* virus is normally defective too, usually at a different locus. It has the capacity to infect the same host but cannot replicate in it. In other words, the two defective virus forms compensate for each other's deficiency and act complementarily. This phenomenon is known as *complementation*.

For example, *rIIA* and *rIIB* are two mutants of the coliphage *T₄*.

Individually they are unable to infect replicate in *E. coli* K_{12} (λ). However, when both of them simultaneously infect K_{12} (λ), infection is successful and plaques are formed. Two genomes *i.e.* that of $rIIA$ and $rIIB$ thus complement each other. Another example is the well known Rous Sarcoma Virus (RSV). Pure preparations of this virus are unable to infect chicken cells. However, when another virus of fowl leukemia group is present as a helper, new RSV particles are formed. The helper virus enables the coat of the virus to be formed which is impossible with RSV alone.

Potato spindle tuber virus (PSTV) is also supposed to be active only in presence of a helper virus.

PHENOTYPIC MIXING

This phenomenon has been shown to be associated with bacteriophage replication. These phages generally are host specific and, as has been mentioned earlier, infection of a host by one of them prevents further infection by phages of the same type. However, other phages, such as close mutants, may infect the same host bacterium.

In such host cells, carrying mixed infections each phage nucleoid synthesises its own protein coat sub-units. During assembly of these sub-units into protein coat around the nucleic acid core, there may be a replacement of one type, partially or totally, by the other type. Thus, the core of bacteriophage *A* could be assembled with the coat of bacteriophage *B*; or its tail may have a fibre of that of *B*. Such apparent alterations are not hereditary and the progeny are not expected to replicate identical particles. Nevertheless, these are otherwise stable and are capable of infecting and lysing susceptible bacterial strains (Fig 6.5 on page 161).

NATURE OF THE VIRAL GENOME

The identity of DNA as the basic hereditary material was confirmed through the classical experimentation on genetic transformation by Avery and his associates (Avery *et al.* 1944). Presence of DNA in viruses, particularly in bacteriophages had been established by then. Subsequently, it was experimentally proved by Hershey and Chase (1952) that phage DNA alone was capable of and necessary for the production of more phage particles. The basic hereditary and biological role of viral DNA was thus found to be identical with that of DNA of bacteria and, possibly that of other living organisms.

The nature of genome in RNA containing viruses remained a

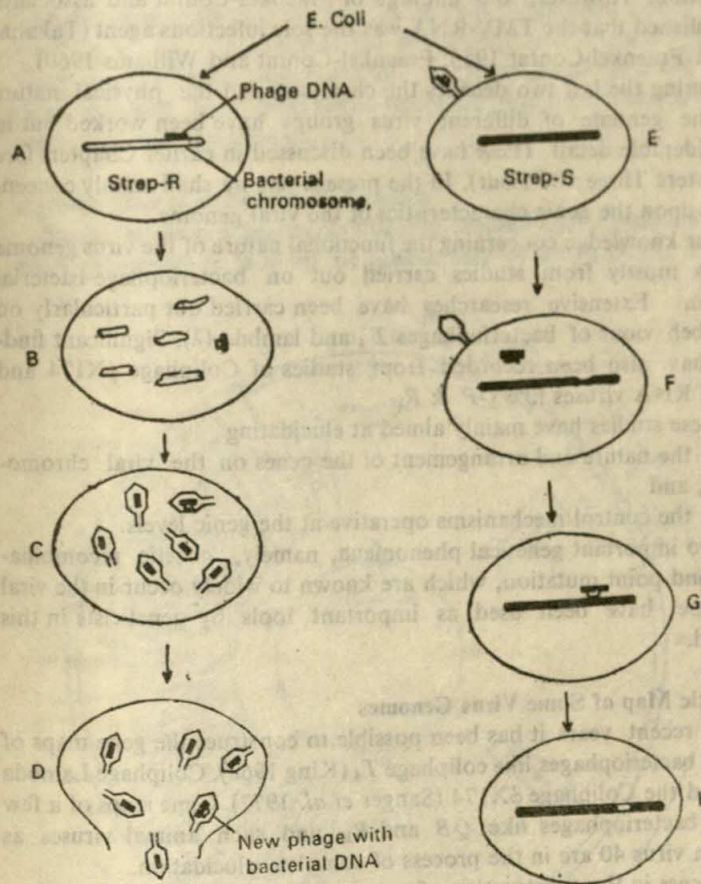


FIG. 6.5. Specialised transduction. This phenomenon is even more rare than generalised transduction. In this event, a particular bacterial strain is suddenly lysed due to the activation of an already integrated phage genome, existing in a temperate state. New particles are formed and are liberated into the medium. When these happen to infect a different bacterial strain, in a few cells at least, they are again integrated with the bacterial chromosome. Thus, a few genes of the first are transferred into the second host cell. As you will note, this does not require prior infection of the first bacterial cell. Characters are accordingly transferred.

puzzle for some time. Tobacco Mosaic Particles (TMV) were, of course, known to be infectious. However, it was not clear whether the TMV-RNA was specifically infectious. It was often observed that the number of TMV particles capable of successful infection was just one

or two per million. It was, therefore, argued that these rare ones probably contained DNA and not RNA, making them capable of infection. However, the findings of Fraenkel-Conrat and associates established that the TMV-RNA was the sole infectious agent (Takahashi & Fraenkel-Conrat 1955; Fraenkel-Conrat and Williams 1960).

During the last two decades the chemistry and the physical nature of the genome of different virus groups have been worked out in considerable detail. These have been discussed in earlier Chapters (see Chapters Three and Four). In the present one we shall mainly concentrate upon the genic characteristics of the viral genome.

Our knowledge concerning the functional nature of the virus genome stems mostly from studies carried out on bacteriophage-bacterial system. Extensive researches have been carried out particularly on the behaviour of bacteriophages T_4 and lambda (λ). Significant findings have also been recorded from studies of Coliphage ϕ X174 and small RNA viruses like QP & R_{17} .

These studies have mainly aimed at elucidating

(a) the nature and arrangement of the genes on the viral chromosome; and

(b) the control mechanisms operative at the genic levels.

Two important genetical phenomena, namely, genetic recombination and point mutation, which are known to widely occur in the viral genome, have been used as important tools by geneticists in this regard.

Genetic Map of Some Virus Genomes

In recent years it has been possible to construct the gene maps of a few bacteriophages like coliphage T_4 (King 1968), Coliphage Lambda (λ) and the Coliphage ϕ X174 (Sanger *et al.* 1977). Gene maps of a few other bacteriophages like QB and R_{17} and such animal viruses as simian virus 40 are in the process of complete elucidation.

Success in the construction of gene maps have been achieved from the analyses of recombination data obtained involving a large number of mutant strains of the various virus species. Further, location of many of genes have been identified by demonstrating that as a result of a particular mutation, the ability to perform one of the steps in phage construction is missing. In this manner, the sequence of events, and consequently the genes, have been worked out in Coliphage T_4 genome.

Another, more-difficult approach towards elucidation of gene maps have been to determine the nucleotide sequences of the genomes (DNA or RNA) of the viruses concerned. Complete sequence analyses

of ϕ X174 (Sanger *et al.* 1977) and R_{17} (Fries *et al.* 1976) have been reported. Utilizing the technique of DNA-RNA hybridization, it has been possible to identify regions of specific nucleotide sequences in these genomes which transcribe for specific *m*-RNA molecules. In other words location of genes could be directly pinpointed. The results thus obtained have been found to corroborate the findings based on recombination experiments.

Genetic Map of Coliphage T_4 Genome

Recombination experiments utilizing thousands of T_4 mutants have made it possible to build up the genetic map of the T_4 DNA. Further,

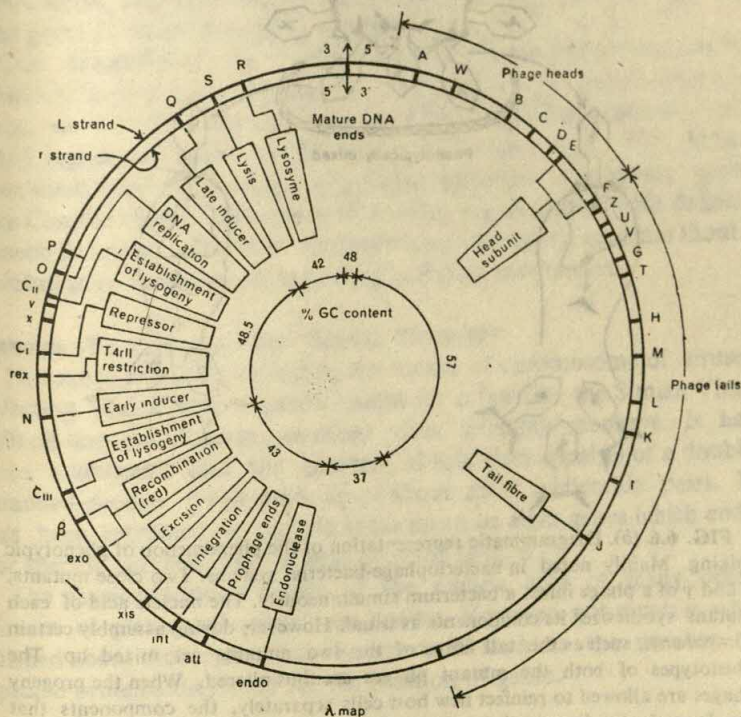


FIG. 6.6 (a). The genetic map of λ showing the function of many of its genes.

with data obtained from studies on deletion mutations (finding out which function/s are ceased in the absence of a particular gene) it has been possible to build up, stepwise, the manner of phage construction (see King 1968). It is estimated that Coliphage T_4 genome consists of 70-100 genes of which a few still remain to be identified. In figure

6.6 (a) the position of the genes involved in various activities of a T_4 phage are outlined.

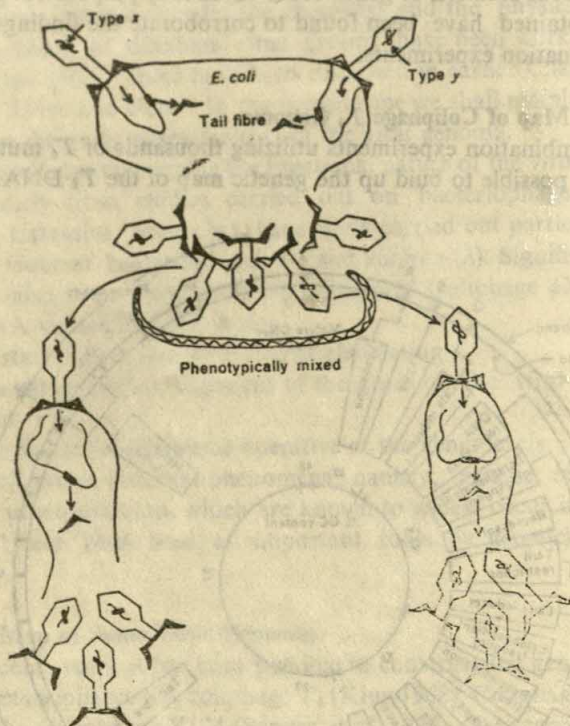


FIG. 6.6 (b). Diagrammatic representation of the phenomenon of phenotypic mixing. Mainly noted in bacteriophage-bacterial systems. Two close mutants, x and y of a phage infect a bacterium simultaneously. The nucleic acid of each mutant synthesizes its components as usual. However, during assembly certain components, such as the tail fibres of the two mutants get mixed up. The phenotypes of both the mutant phages are thus altered. When the progeny phages are allowed to reinfect new host cells separately, the components that are formed, are in accordance with the nucleic acid core. These get assembled into virions that are of the pure types. Thus, phenotypic mixing is not a genetical phenomenon.

Coliphage Lambda (λ) Genome

Figure 6.6 (b) depicts the genetic map of Coliphage (λ). Information obtained from studies on recombination and deletion mutations has made it possible for phage geneticists to construct the genetic map of this phage. It has been established that there are about fifty genes comprising the (λ) genome. In lytic state all these genes are

sequentially functional. However, in the lysogenic state, most of these genes remain repressed, possibly because of the activity of a repressor gene. Only specific conversion genes remain functional.

Nature of ϕ X174 Genome

Complete genetic analysis has been possible in the single stranded DNA containing Coliphage ϕ X174. Genetic map of this phage has been constructed on the basis of classical experiments on mutation and recombination. Some gene products and their corresponding gene loci have been tentatively identified by means of the technique of DNA-RNA hybridization. It has been estimated that this phage has nine genes arranged as a circular strand.

One intriguing aspect of the genome of this phage has been its relatively large genetic potential as compared to its small structure (mol. wt. 1.7×10^6). Recently done complete sequence analysis of this DNA suggests an answer to the riddle (see Sanger *et al.* 1977). It has been established that certain sequences represent overlapping genes (see Chapter three). Occurrence of overlapping sequences with definite genetic function, it found in prokaryotic or eukaryotic genomes should revolutionize our concepts regarding recoding mechanism.

Genome of a Polyoma Virus (Simian Virus 40)

Not much is known regarding the nature of chromosome of viruses infecting plants and animals, except for a few like the Simian Virus (40) an oncogenic (cancer causing) virus infecting monkeys. It has been established that the genome of this virus consists of a double stranded circular DNA made up of about 5500 nucleotide pairs. It has been estimated to contain some seven or eight genes which code for specific enzymes and for coat proteins (Fig. 6.7).

The physico-chemical nature of the nucleic acid of many other viruses of plants and animals are known. However, not much is established about their functional characteristics such as the number of specific proteins they code for the position of genes.

Control of Gene Functions

Manifestly, the virus genome is the simplest of all. Nevertheless, the mechanisms governing its functions are far from clear. While active, the virus genome generally functions at two levels. First, it gains an overall control over the host genome, literally switching the latter off. Secondly, at the same time it programmes sequentially and chronologically for the synthesis of various virus components and arranges their assembly into mature particles. To cap it all, it utilizes the host's

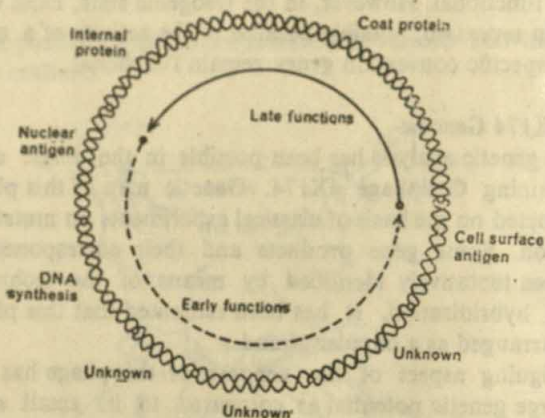


FIG. 6.7. The genetic structure of polyoma (SV 40). Still to be determined are the relative location and sizes of the various genes. The existence of 8 distinct genes is postulated. If the protein products are smaller than with other known genes, the gene number will be correspondingly larger.

metabolic machinery for the purpose. At other times, the genome itself remains repressed and inactive as a prophage or a latent virus. The nature of genetical control over all these processes are beginning to be elucidated.

To know precisely as to how the control by the T_4 genome is exercised over the host genome, one has to understand the role played by the enzyme RNA polymerase. It may be recalled that subsequent to viral infection three fundamental changes occur in the host at the metabolic level. These are:

- (a) cessation of transcription of the host genome.
- (b) initiation of transcription of the viral genome.
- (c) sequential transcription of the viral genome.

All these metabolic processes involve the host-cell RNA polymerase. This enzyme is made of five subunits. Each subunit is structurally a polypeptide chain. These subunits have different molecular weights and are referred to as β' , β , σ , a and w . The subunit a is present twice. Out of these five β , β , a and w aggregate with each other more firmly than they do with σ . It is, therefore, easy to isolate $\beta' \beta a a w$ aggregate, known as the *core enzyme*. The core enzyme is able to catalyze the formation of polyribonucleotide with or without the factor σ (sigma).

Importance of sigma (σ) factor lies in its ability to recognize (1) the proper strand of the DNA to be transcribed and (2) a specific nucleotide sequence of the DNA. Coding for initiation of an RNA chain.

In the absence of these two vital recognitions, the functional transcription of the gene is unattainable.

One explanation to account for the three post-infection changes mentioned earlier would be

(1) Segregation or blocking of σ factor from the core enzyme thus rendering proper transcription of host gene impossible.

(2) Synthesis of virus specific σ factors which would enable the host RNA polymerase to recognise virus gene strands and sequences.

(3) Synthesis of different virus specific σ factors accounting for synthesis of different viral proteins chronologically and sequentially.

In recent years evidence have been accumulating largely substantiating the above contentions. For instance, it has been established that

(i) The host specific σ is unable to associate with the core RNA polymerase due to changes occurring in the structure of the core enzyme itself.

(ii) Immediately after infection, the virus (T_4) genome is known to synthesize virus specific σ factors. For this a few genes of the virus are capable of utilizing host σ factor.

(iii) The sigma factor synthesized in the beginning help in the synthesis of early proteins. Later during the life cycle a second type of σ factor is synthesized which enables the transcription of genes responsible for late proteins.

More recently, Stevens (1972) reported that four T_4 proteins get non-covalently attached to *E. coli* RNA polymerase after T_4 infection. Subsequent studies revealed that each of them are involved in the control of synthesis of major classes of T_4 RNA at different stages during T_4 life cycle. Apparently all of them interfere with transcription of the phage DNA. This control is exercised by altering or inactivating sigma factors of *E. coli* RNA polymerase. For instance one protein, about 10,000 daltons, has been reported to inactivate the sigma factor of the enzyme (Stevens & Rhoton 1974).

Evidences suggest that another subunit of about 15,000 daltons prevents specifically the transcription of cytosine containing DNA (Sirotkin *et al.* 1976). The other two subunits have been demonstrated to be needed for synthesis of T_4 RNA appearing late after infection, presumably linked with synthesis of late proteins. Apparently these two are specific sigma factors for the concerned RNA polymerases.

Genetic Control in Coliphage Lambda (λ)

As has been mentioned earlier, coliphage λ is capable of offering both lytic and lysogenic responses. In the former the λ genome becomes normally expressed. Thus the host genome is switched off, λ DNA

synthesised, heads and tails are formed and ultimately assembled into a fresh crop of λ particles, resulting into lysis of the host cell.

In the lysogenic response, the host cell survives, and the circular DNA of the phage is linearly inserted into the bacterial genome. At the same time it synthesises the repressor which blocks all lytic functions. Thus the lytic death of the host cell is prevented and a symbiotic association which is passed on vertically (from one generation to the next) is established. This can be reversed on induction.

Recent researches have further elucidated the nature of control during

(a) lysogenic (prophage) state.

(b) induced lytic state.

It has been found that during prophage state only one operon (cluster of genes) is transcribed. As a result only those repressor proteins are produced which totally inactivate the rest of the prophage genome. This is possibly achieved owing to non-recognition of these operons or their promoters by the host cell RNA polymerase. Additionally, the host cell is provided immunity against further infection against similar phages.

Induction leads to conversion of the prophage to lytic state. This phenomenon is initiated with inactivation of the repressor and detachment of the inserted linear genome from the host chromosomes to form the original circular one.

Inactivation of repressor leads to the normal functioning of the rest of the phage operons. The orderly and sequential transcription of these operons leads to the production of a crop of progeny phage particles. Apparently the block at the transcription level is released.

Control of SV 40 Genome

Control of activities of SV 40 genome is either lysing or transforming host cells has been attributed to synthesis of virus specific *T*-antigen. In a recent report (Weil *et al.* 1976) it has been suggested that the *T*-antigen polypeptides are synthesised by the viral genome. The *T*-antigen in its turn governs the activity of most other effects of SV 40 genome. However, whether it acts as a repressor or as a modifier of the host cell RNA polymerase activity is not yet known.

Chapter Seven

PHYLOGENY AND TAXONOMY OF VIRUSES

Phylogeny of an organism usually refers to its evolutionary history. More specifically, it relates to the biological relationship one organism has with the others. Phylogeny, therefore, cannot be considered by taking up one particular organism in exclusion to the others. And above all, any discussion on phylogeny makes it imperative that the subject under consideration is a member of the living world. On both these counts any consideration of the phylogeny of viruses would seem paradoxical and even questionable, to say the least, if not downright blasphemous; for, we are not yet sure as to what viruses biologically are. Nevertheless, during the course of our discussions in the following paragraphs, we would endeavour to present the various viewpoints on the subject that have been put forth from time to time. While the purpose of all these have been to resolve the riddle concerning the biology of viruses, the problem remains as formidable, alas, as it had been earlier.

DEFINING A VIRUS

Let us proceed on the hypothesis that viruses have an important position in the biological system of this planet, even if they are strictly speaking not to be considered members of the living world. How are they to be defined if we are to set them apart from the rest of the world, both animate and inanimate?

Attempts have been made from time to time to define a virus according to the information available. One such early definition, that of Bawden (1964) considers viruses as, "submicroscopic, infectious entities that multiply only intracellularly and are potentially pathogenic." Another definition that of Lwoff (1957) says, "Viruses are infectious, potentially pathogenic nucleoprotein entities, with only one type of nucleic acid, which reproduce from their genetic material, are unable to grow and divide and are devoid of enzymes." There have been similar other pronouncements all of which have been considered

inadequate somehow (see Hatton 1964).

Inadequacies regarding framing an overall acceptable definition of viruses were, therefore, quite apparent. In fact it was found that viruses could be considered from another angle as well *i.e.* the angle of an inanimate. Crick and Watson (1956) suggested a perfect definition of at least the small viruses which could fit perfectly into the definition of molecule as "a particle all of whose atoms are arranged in a more or less constant relationship to each other." The futility of looking at viruses with a strict definition was then quite clear. The pronouncements made in a lighter vein like "*viruses are viruses because they are viruses*" (Lowff 1966) and that "it would perhaps have satisfied Beijerinck to say that they are creatures endowed with life when drawn into a living cell" (see Waterson 1968) are an indication of the situation.

Thus, while viruses, strictly speaking, could not be defined, they could surely be set apart from other organisms on the basis of certain discriminative characters they possessed. These have been listed by Lwoff and Toournier (1971) as follows:

- (i) They are all potentially infectious;
- (ii) Presence of a single nucleic acid;
- (iii) Incapacity to grow and divide;
- (iv) Reproduction from the genetic material only;
- (v) Absence of enzymes for energy metabolism (Lipmann System);
- (vi) Absence of ribosomes;
- (vii) Absence of information for the production of enzymes in the energy cycle;
- (viii) Absence of information for the synthesis of ribosomal proteins;
- (ix) Absence of information for the synthesis of ribosomal RNA and soluble (transfer) RNA.

Lwoff and Toournier suggest that there is a definite correlation between all these characteristics and that presence of any one of the characters (from character *ii* to *viii*) is sufficient to establish that an "infectious particle belongs to the viral world." These characters clearly set them apart from other micro-organisms (Table 7.1).

Considering all these aspects, we can say that definition of the virus by itself is not important. What matters more is the establishment of definite marker characters which distinguish them from the world of the rest of the living organism yet not putting them out of it. Acceptance of viruses as 'living' has been slow to come but it is bound to come, because it is becoming increasingly clear that an attempt to define viruses without defining categorically life itself would be an exercise in futility. No separate definition is really necessary. All we can hope to do is to characterize them as far as possible.

TABLE 7.1. DISCRIMINATIVE CHARACTERS OF VIRUSES AND OTHER MICRO-ORGANISM

| Characteristic | Viruses | Others (Protozoa or Bacteria) |
|--|------------|-------------------------------|
| Nucleic Acid | DNA or RNA | DNA and RNA |
| Reproduction from the sole genetic material | + | 0 |
| Growth | 0 | + |
| Division | 0 | + |
| Information for the synthesis of respiratory enzymes | 0 | + |
| Presence of respiratory enzymes | 0 | + |
| Information for the synthesis of transfer RNA | 0 | + |
| Information for the synthesis of ribosomal RNA | 0 | + |
| Presence of ribosomes | 0 | + |

(Adapted from Lwoff and Tournier 1971)

ORIGIN OF VIRUSES

As mentioned earlier, viruses have been regarded as independent genetic units because of their ability to (i) multiply according to a specific genetic programme; and (ii) adapt successfully to newer host systems. Presence of a well elaborated code in the virus genome, often extending upto thousands of nucleotides shows that these are too complex to have originated *de novo*. Therefore, one is logically lead to the supposition that viruses must have originated from some free living organism. It has been contended that a phase of regressive evolution must have appeared sometimes during the evolution of life on this planet leading to the development of virus and virus like entities.

How all these might have happened remained and still remains a matter of speculation. An interesting hypothesis put forth by Lwoff and his associates in this regard suggests that some primitive micro-organism might have become, during the dawn of evolution, parasitic on some other micro-organism for a few essential metabolites like amino acids and monosaccharides. Initially they were able to obtain these simple requirements extracellularly. Later on, their requirements became more and more exclusive. Complex essential molecules that could not be assimilated extracellularly (because of their inability to come out of the cell membrane) began to be required. These molecules could be obtained only intracellularly, a situation that leads to the organisms becoming intracellular parasites.

Such extreme form of parasitism leads to gradual loss of essential synthetic enzymes and ultimately onto a phase analogous to that of present day viruses. This theory is referred to as the *Regressive Theory* of the origin of viruses. Although this hypothesis had evoked considerable attention and interest in the past, several objections have been raised against its validity in recent years. The principal amongst these are the non-existence of transitional stages and the noncellular organisation of the viruses.

Another theory explaining the origin of viruses postulates that these are evolved as a result of accidental escape of cell organelles from mature cells. Presence of DNA of a type different from the nuclear DNA in such cell organelles as the chloroplast and the mitochondria has lead many to consider these cell organelles as independent replicating entities. It has also been contended that these organelles have definite potentialities to exist as independent units outside the cell of which they are a part. And also that they might have escaped the cellular environment and come to exist independently in some remote past. Later, these became parasitic in an attempt to survive under conditions which subsequently became unfavourable. The escapee organelles had in the meantime changed a lot and their forced intracellular parasitic existence led to the development of viruses.

Another similar hypothesis suggests the escape of bacterial genome fractions or *Episomes* and their subsequent induction into new host cells. Episomes have been defined as particles with ability to replicate in either the autonomous or the integrated state and to shift between these alternative states. Further, these are capable of being transferred from cell to cell. These traits lend credence to the view that viruses might have originated in this manner. Existence of stable gene products and their self-replicating copies in cells of other organism, such as bacterial plasmids and metagons in *Paramecium*, further strengthens the idea. It has been contended that when such genomic fragments become infectious, and could spread from one host to another, viruses came to be borne.

Expressing an alternative view Professor Martin Pollock (1970) suggested that cell genomes themselves are *mosaics* that have grown by incorporating a variety of smaller particles and that such particles with limited biological capabilities evolved independently sometimes during the evolution of life. Implicit in this statement is the idea that viruses are a group of such independently originating particles. A similar hypothesis proposed by Altenberg in 1946 envisaged the evolution of viruses as a corollary to the attempts towards cell formation. This concept failed to get acceptance because this could not explain

the occurrence of genetic complexity in viruses.

Phylogeny of viruses thus remains obscure, as yet. But one thing has become quite clear. We may now say that whatever be their mode of appearance on this planet, whether as an end product to a process of gradual retrogression or as independent entities originating *de novo*, there exists a unique and exclusive relationship between a virion and its host. In the proper understanding of this relationship lies the answer to the riddle of the biological status of these particles.

However, whether independently originating or not, viruses are regarded by some as transmitters of information carrying genetic material or as Smith (1974) puts it as "bits of infectious heredity in search of a chromosome."

BIOLOGICAL STATUS OF VIRUSES— ARE THEY ANIMATE OF INANIMATE?

Viruses have remained a biological enigma. We have never been sure about their true status as a biological entity. Nor are we sure whether these are living or non-living for that matter. They exhibit a few characteristics typically of inanimate nature. Though, on the other hand, they also show some properties of the animate as well. This naturally makes the picture quite confusing.

Of the traits exhibited by viruses and which are associated with the inanimate, the fundamental one is their ready crystallisability. W.M. Stanley's pioneering success in crystallising tobacco mosaic virus (TMV) and subsequent crystallization of various other viruses of plants, animals, bacteria and a few other groups of organisms by different workers, clearly demonstrated that these agents were distinct from the living organisms in this very important respect.

Apart from this, some other characteristics of viruses associable with the inanimate are, for instance, that they sediment on ultra-centrifugation according to their molecular weights and that they exhibit disjunction on treatment with detergents into their component parts. Also they are totally dependent upon living organisms for their very existence and are incapable of acting on their own, they also lack any energy producing energy system (Lipmann System), a must for a living system.

On the other hand, their similarities with the living world are not few. The most striking resemblance lies in their capacity to multiply number of the same genetic type. Of course, they cannot multiply without the assistance of a living organism but the process is undoubtedly analogous to the phenomenon of reproduction in the established

living organisms. The other important biological trait is their extremely specific intracellular parasitism, a property universally associated with living organisms. The host is usually specific which points towards a biological relationship of a nature that could only be associated with living organisms.

Further, the viruses are mutable, *i.e.* their characters can be altered permanently and hereditically. Their irritability is again a trait of the living. They can be reconstituted specifically from previously separated components exhibiting specific assembly and individuality of formation. Viruses also show the capacity to bring about enzymatic changes *in vitro*. They have the ability to infect and can be transmitted from one host to the other. Indeed, they are known to outlive their host cells and adapt themselves to new hosts, sometimes of a completely different biological nature. In other words, viruses may even be attributed with a certain degree of independence. Their capacity to be expressed as a part of the host genetic system, as for example, with the lysogenic bacteria or with C-type RNA virus infected vertebrate cells is another interesting trait pointing towards an association analogous to that between living organisms.

It is quite evident, therefore, that while it is difficult to ignore viruses as just another manifestation of the inanimate world, it is not easy to define them within the accepted framework limiting living organisms either. On the face of it, when we evaluate viruses on the basis of the prevalent definitions of life, we have to conclude that they are perhaps non-living. Professor Andre Lwoff defines an organism as an "independent unit of integrated and interdependent structures and functions." This framework would obviously exclude viruses, as they are totally dependent upon some living host and are no more independent than the cellular macromolecules.

But if we were to slightly modify the definition of life, accommodation of viruses into the living kingdom could easily be accomplished. And if we were to accept Oparin (Oparin 1965) that the inanimate and the animate worlds are merely the parts of the same evolving system representing different phases in the same evolving process, all this controversy would just vanish into the thin air. Therefore, it may be asserted that even if the issue remains somewhat controversial, viruses definitely have a very important place in the biological system of this planet. These could be regarded as something unique, representing a line of development which did not flourish. In this context, Lwoff's contention that viruses should be considered as viruses because *viruses are viruses* becomes quite significant instead of appearing merely funny.

CLASSIFICATION OF VIRUSES

The biological status of viruses has remained uncertain. However, as more and more different types of viruses came to be recognised, the necessity to categorize and classify viruses was increasingly felt. In the earlier days of virology, when the number of known virus types was comparatively smaller, the classification systems used were *ad hoc* and arbitrary, based upon one or two easily recognisable characters only. With the increase in information about the form and function of viruses, more and more specific traits began to be considered for the purposes of classification. Further, characters instead of being considered arbitrarily and independently of each other, were now increasingly being considered in a correlated manner. Also, over all affinities amongst virus groups became an important criterion.

In recent years, several systems of classification have been proposed (cf. Lwoff *et al.*, 1962; Bellet, 1967; Gibbs, 1969). All of them have proved to be controversial and have failed to find general acceptance. For one thing, these systems have not been based on a common set of principles. Besides, most of these, except perhaps the one proposed by Lwoff and his associates, are restricted to one virus group or the other.

To resolve the problem, a separate agency, the International Committee on Nomenclature of Viruses (ICNV) was set up at the International Congress for Microbiology held in Moscow in 1966. Its job was to look into the various aspects of classification and nomenclature of viruses and to devise universally acceptable norms for both. The ICNV set up different sub-committees to look into various broad groups of viruses and devise systems of classification for them on the basis of a uniform set of principles. The ICNV specifically laid down that:

- (i) groups (or genera) of viruses must be defined and listed;
- (ii) species belonging to these genera be listed;
- (iii) names for the groups be provided;
- (iv) taxonomic development in various broad branches of virology be summarised and be based on uniform set of principles;
- (v) norms for description and identification of viruses be set.

The work of the various sub-committees is in progress. Certain guidelines regarding classification have also been provided. However, not much headway has been made towards devising uniform system of classification. Positions taken by virologists working in different fields are still to be reconciled.

Early Practices

A normal early practice has been to categorise viruses on the basis of their natural hosts. Accordingly, viruses were classified as under:

(a) *Plant viruses*. The group included particles infecting plants exclusively. Plant viruses could be further subdivided into sub-groups like (i) bacterial viruses; (ii) fungal viruses; (iii) algal viruses and so on.

(b) *Invertebrate viruses*. Those infecting arthropoda and some other invertebrate phyla.

(c) *Vertebrate viruses*. Those infecting vertebrates. These could be subdivided into:

(a) Cold blooded vertebrate viruses; and

(b) Warm blooded vertebrate viruses.

(d) *Dual host viruses*. Viruses parasiting upon two different hosts. Such viruses could be further categorized thus:

(i) Arthropod-plant viruses; and

(ii) Arthropod-warm blooded vertebrate viruses.

Another early system to be proposed was in the Bergey's Manual of Determinative Bacteriology (7th edition). Here the viruses have been considered to be bacteria like micro-organisms and an ordinal rank was proposed for them, namely, the order *Virales*. This order was further subdivided into three suborders, namely the Phaginae (infecting bacteria), Phyto-phaginae (infecting plants) and Zoo-phaginae (infecting animals). In subsequent editions, however, viruses have been excluded from any such consideration.

Such early systems of classification were found to be inadequate. First, they were not based on even a majority of information available subsequently. Secondly, these systems were based on one arbitrarily selected character, namely, the nature of the host. Viruses, therefore, tended to be classified into groups and subgroups in accordance with the host type. This soon led to confusion because a large number of other characters were not taken into consideration.

Modern Approaches

Modern systems of classification are based primarily upon accurate information about the physico-chemical characteristics of the viruses. Biological traits such as host type and the nature of specificity are utilised as secondary bases. Of the several systems proposed on the above lines, the one by Professor Andre Lwoff and his colleagues Drs Horne and Toournier (the LHT system) was proposed in 1962. Later, after it was modified in 1966 was accepted in many quarters in its modified form.

LHT system. This system is based upon the precise consideration of five major characteristics of the viruses and their priority wise evaluation. These traits are referred to as the 'Essential Integrants.' These are the following in order of importance:

1. The type of nucleic acid
2. Symmetry of particles
3. Presence or absence of envelop around the nucleocapsid
4. Diameter of the helical nucleocapsid and/or
Number of capsomeres in the cuboidal types.

These traits were utilized singly or in correlated combinations to classify viruses into groups, sub-groups and infra sub-groups. The concept of hierarchical arrangement of more and more inclusive groups was sought to be established.

Using these characteristics viruses have been classified upto families. A broad outline of the scheme is presented below.

Phylum Vira: consisting of particles accepted to be viruses.

Sub Phylum Deoxyvira: viruses with DNA.

Class Deoxyhelica: DNA containing helical viruses.

Order Chaetovirales: viruses with envelop.

Family: pox viridae.

Order enidovirales: viruses without envelop.

Family: inidoviridae.

Class Deoxycubica: DNA containing cubical viruses.

Order Peplovirales: with envelop.

Family: Herpesviridae.

Order Haplovirales: without envelop.

Family: Microviridae, Parvoviridae, Densoviridae, Papilloviridae, Adenoviridae and Iridoviridae.

Class Deoxybinales: DNA containing binial viruses.

Order Urovirales: without envelop.

Family: Phagoviridae.

Sub Phylum Ribovira: viruses with RNA.

Class Ribohelica: RNA containing helical viruses.

Order Rhabdovirales: without envelop.

Family: Dolichoviridae, Protoviridae, Mesoviridae, Leptoviridae, Adroviridae.

Order Sagovirales: with envelop.

Family: Myxoviridae, Paramyxoviridae, Stomatoviridae, Thylaxoviridae.

Class Ribocubica: RNA containing cubical viruses.

Order Gymnovirales: without envelop.

Family: Napoviridae, Reoviridae.

Order Togovirales: with envelop.

Family: Arboviridae, Encephaloviridae.

Classification at generic and subgeneric levels is based on such determinants as:

- (1) Primary structure of nucleic acid (base composition nearest neighbour frequency, base sequence, homology, etc.)
- (2) Molecular weight; antigenic properties of the capsid
- (3) Organisation of the nucleocapsid
- (4) Specificity for host
- (5) Mode of development
- (6) Virulence
- (7) Clinical effects.

It must be emphasised here that the traits mentioned above are not all used, rather a combination of the relevant factors is decisive.

The LHT system has evoked considerable interest amongst virologists. It has been applauded because (i) it is the first attempt to classify viruses as a whole, (ii) it is based on the structure and composition of viruses, (iii) it attempts to classify on the basis of correlation amongst characters.

However, the scheme has also been widely criticized. Wildey (1962) noted that viruses were a heterogeneous assemblage of entities and, therefore, any classification of viruses in a hierarchical pattern was likely to be artificial. Gibbs (1969) calls the system arbitrary as it gives weightage to a few chemical and physical characters whose selection is not based on proper evaluation. Bellet (1967) suggested that the LHT system was artificial as it did not attempt to take affinities amongst different groups into consideration.

Despite criticisms, the LHT system has been getting more and more attention in recent years. It has been noted that limitations notwithstanding the system remain the only comprehensive one to date. One might add that any system of classification, by the very nature of the job, tends to become arbitrary to a certain extent and, therefore, it is better to judge a system on the basis of its usefulness in classifying a group of organism. From this point of view the LHT is a significant contribution.

Bellet's system. Bellet (1967) proposed a system for classifying viruses. It was based on two physico-chemical characteristics namely the molecular weight and the percentage guanine+cytosine content of the viral nucleic acids. Additionally, antigenic or serological reactions and phenotypic properties were also taken into consideration. The system of classification was devised on the basis of affinity coefficients existing amongst the various groups examined on the basis of the nucleic acid characteristics. These co-efficients were determined by feeding the preliminary data into computers.

Accordingly the viruses are divided into two major subgroups namely, the viruses with single stranded and those with double stran-

ded nucleic acids. The coefficient of similarities of the various virus groups belonging to these major subgroups were then computed. More closely related subgroups were then clumped together.

The approach has been new. However, the system could not be used as independent of categorization based on phenotypic properties. In fact, where even the computer data did not correspond to the phenolytic behaviour, Bellet has been cautious in his approach and often did not accept the computer data. The system corroborated much of LHT system. However, this system restricts itself mainly to animal viruses and can, therefore, be only of limited use.

Gibbs system. Gibbs in 1969 proposed a system of classification for plant viruses. As a basis for his system, he took into consideration (i) the shape of the capsid *i.e.* whether isometric or anisometric, (ii) the mode of transmission, (iii) the type of vector, (iv) the symptoms of infection, and (v) the nature of the accessory particles. Accordingly, he grouped some 135 known plant viruses into 6 broad groups or clusters. These broad groups were further categorized into subgroups showing a higher degree of inclusiveness.

This system did help in categorising plant viruses on the basis of information available on them, whatever may be the basis for such categorisation. However, it came in for a lot of criticism as it did not take into account such well established physico-chemical criteria as the symmetry of the capsid and the nucleic acid type. However, in recent years, Gibbs has modified his criteria by incorporating the nucleic acid nature as well. Some of the groups formulated by Gibbs have been accepted as stable taxonomic group by the ICNV (*see* Wildey 1971).

DESCRIPTION AND IDENTIFICATION OF A VIRUS

Gibbs and associates proposed in 1966 that all viruses be technically identified on the basis of certain approved parameters. They suggested that the following eight characters could be used for the purpose:

- (1) The nucleic acid type;
- (2) The number of strand in a nucleic acid;
- (3) Its molecular weight;
- (4) Percentage of nucleic acid in a virion;
- (5) The form of the particle;
- (6) The form of the nucleocapsid;
- (7) The host; and
- (8) The vector.

They further suggested that these parameters be defined by abbre-

viations and that the abbreviations presented in the form of a formula describing a virus. They renamed such formulae as *Cryptogram*.

This proposal was accepted with certain modifications by the ICNV. Cryptograms for a large number of viruses were drawn and published. Some of them are listed in Table 7.2. Use of cryptograms has increased in recent years.

TABLE 7.2

| Virus | Cryptogram | | | |
|--------------------------|------------|-----------|----------|----------|
| | 1st Pair | 2nd Pair | 3rd Pair | 4th Pair |
| Pox virus (vaccinia) | D/2 | 160/5-7.5 | X/* | V/0 |
| Coliphage T ₄ | D/2 | 130/40 | X/X | B/0 |
| Herpes Simplex | D/2 | 68/7 | S/S | V/0 |
| Coliphage ϕ X174 | D/1 | 1.7/25 | S/S | B/0 |
| Tobacco Mosaic | R/1 | 2/5 | E/E | S/0 |
| Turnip Yellow Mosaic | R/1 | 1.9/37 | S/S | S/CI |
| Tobacco Necrosis | R/1 | 1.5/19 | S/S | S/fu |
| Cauliflower Mosaic | D/2 | 5/15 | S/S | S/Ap |
| Yellow Fever Virus | R/* | */* | S/* | V, I/Di |
| Poliovirus | R/1 | 2.5/30 | S/S | V/0 |

Abbreviations: 1st Pair NA type/Strand (D/R; 2/1)
 2nd Pair Mol. wt (10^6) of NA/Percentage
 3rd Pair Outline of particle/nucleocapsid
 [Spherical (S), Elongated (E), Complex (K)]
 4th Pair Host/Vector Bacterium (B), Seed plant (S)
 Invertebrate (I), Vertebrate (V)
 Diptera (Di), Coleoptera (CI)
 Aphid (Ap), Fungus (Fu)

* Unknown

Phanerogram

Another way to identify viruses on the basis of symbolic description was proposed by Lwoff and Tournier (1969). It uses four parameters, namely Nucleic acid type, Symmetry of capsid, Naked or enveloped nature of the nucleocapsid and number of capsomers/diameter of nucleocapsid. They called the formulae obtained on the basis of these parameters *phanerograms*. The approach was similar to that for cryptogram formulation.

NOMENCLATURE OF VIRUSES

No system of classification can be fool proof without assigning definite nomenclature to the various hierarchical categories. In recent years there has been a consensus amongst virologists to accept and

use the various categories used in biological nomenclature of organisms, though some virologists still prefer to use the term 'group.'

The International Committee for Nomenclature of Viruses established rules guiding the nomenclature of viruses. Some of these are:

- (1) The species includes identical viruses.
- (2) The genus is a group of species having common characteristics.
- (3) The name of the genus must terminate in the suffix virus and an effort should be made towards binomial nomenclature.
- (4) Each genus must have a type species.
- (5) A group of genera are to be referred to as a family which shall have a name terminating in *idae*.

These rules have been used and nomenclature of virus groups suggested. However, many eminent virologists are still sceptical about adoption of binomial nomenclature for viruses. For instance Gibbs (1967) has pointed out no definite agreement is there as to how to define a species and that in the absence of an acceptable definition of a species, no nomenclature would be valid. Nevertheless, acceptable binomials of many viruses have been proposed. Table 7.3 lists the binomials of certain important viruses.

TABLE 7.3. BINOMIALS FOR SOME IMPORTANT VIRUSES

| <i>Virus</i> | <i>Binomial</i> |
|----------------------|------------------------------------|
| Pox (Variola) | <i>Poxvirus variolae</i> |
| Polyoma | <i>Polyomavirus neoformans</i> |
| Herpes | <i>Herpesvirus hominis</i> |
| Phage T ₂ | <i>Phagovirus (coli) tsecundus</i> |
| Tobacco Mosaic | <i>Protovirus tabacci</i> |
| Influenza | <i>Myxovirusinfluenzae</i> |
| Rabies | <i>Rabiesvirus canis</i> |
| Polio | <i>Poliovirus primus</i> |
| Arbo | <i>Arbovirus occidentalis</i> |

CHARACTERISTICS OF SOME VIRUS GROUPS

In its first report the "International Committee on Virus Nomenclature" presented 45 well delineated and approved groups of viruses. These groups are the following:

1. Pox virus group
2. T-even phages
3. Iridescent virus group
4. Nuclear polyhedrosis virus group and granulosis virus
5. Herpes virus group
6. Lambda (λ) phage
7. Adenovirus group
8. Cauliflower mosaic virus group
9. Papilloma virus group

- | | |
|--|--|
| 10. Polyoma virus group | 28. Tobacco Rattle virus group |
| 11. Lipid phage PM2 | 29. Nepovirus |
| 12. Parvovirus group | 30. Tobacco mosaic virus group |
| 13. ϕ X group | 31. Turnip yellow mosaic group |
| 14. Filamentous phage group | 32. Tomato bushy stunt virus group |
| 15. Reovirus group | 33. Tobacco Necvinlis virus group |
| 16. Cytoplasmic polyhedrosis virus group | 34. Pea enation mosaic virus group |
| 17. Leukosis virus group | 35. Bromemosaic virus group |
| 18. Para-Myxovirus | 36. Ribophage group |
| 19. Cowpea mosaic virus | 37. Cucumber mosaic virus group |
| 20. Orthomyxovirus group | 38. Potato virus Y group |
| 21. Alfalfa mosaic virus | 39. Carnation latent virus group |
| 22. Vescicular Stomatilis virus group | 40. Potato virus X group |
| 23. Arbovirus Group A | 41. Infection bronchitis virus group |
| 24. Arbovirus Group B | 42. Tomato spotted niff virus |
| 25. Vescicular exanthema virus group | 43. Lymphocytic choriomeningitis virus group |
| 26. Rhinovirus group | 44. Papovirus group |
| 27. Enterovirus group | 45. Enterovirus group |

The grouping together a number of viruses at the same time separating them from other similarly demarcated groups was done primarily according to nucleic acid and capsid characteristics of the virus particles. The kinds of the host and specific vector, if any, were also taken into consideration. Some of these groups were given generic ranks and approved generic names. A few were given family ranks and names. Names and taxa of a few others are still to be proposed and/or approved. We shall reproduce here some of the approved groups presented by the ICNV.

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|------------------------------|--|------------------------------------|-------------------------------|
| <i>Genus</i> | <i>Poxvirus group</i> | <i>Poxvirus</i> | $(D/2 : 160/5-7.5 : X V^*:0)$ |
| <i>Type species</i> | <i>Vaccinia virus</i> | — | $D/2 : 160/5 : X ^* : V/0$ |
| <i>Main characteristics:</i> | Contain 5 to 7.5% double-stranded DNA, mol. wt. 160×10^6 . G+C 35 to 40%. Brickshaped or ovoid complex particles 170 to 250×300 to 325 nm, about 5,000S, BD (CsCl) 1.1 to 1.33 g/cm. Several layers with lateral bodies \pm envelope. Characteristic surface patterns. Probably contain enzymes, e.g., RNA polymerase. NP antigen common to all members. All members exhibit non-genetic reactivation. Five subgroups. Members of subgroups A to E also have other antigens in common and can recombine genetically. Multiply in cytoplasmic foci. | | |
| <i>Other members:</i> | Cowpox virus | $D/2 : 160/5 : X ^* : V/0$ | |
| | Orf virus | $D ^* : * / 7.5 : X ^* : V/0$ | |
| | Sheep pox virus | $* ^* : * ^* : X ^* : V/0$ | |
| | Fowlpox virus | $D/2 : 200/^* : X ^* : V/0$ | |
| | Myxoma virus | $(D)^ ^* : * ^* : X ^* : V/Di, Si$ | |

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|------------------------------|---|----------------------|----------------------------|
| <i>Genus</i> | <i>T-even phages</i> | — | (D/2 : 137/40 : X/X : B/0) |
| <i>Type species</i> | <i>Coliphage T4</i> | — | D/2 : 137/40 : X/X : B/0 |
| <i>Main characteristics:</i> | Contain a single molecule of double-stranded DNA, mol. wt. about 130×10^6 , about 55 nm long, contain hydroxymethyl cytosine, G+C content about 34%. Particles have a head diameter about 100 nm and a complex contractile tail about 100 nm long. | | |
| <i>Other members:</i> | <i>Coliphage T2</i> | | D/2 130/40 : X/X : B/0 |
| | <i>Coliphage T6</i> | | |

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|------------------------------|--|----------------------|-------------------------------------|
| <i>Genus</i> | <i>Nuclear polyhedrosis virus group and granulosis viruses</i> | <i>Baculovirus</i> | (D/2 : (80-100/8-15) : U/(E) : I/O) |
| <i>Type species</i> | <i>Bombyx mori nuclear polyhedrosis virus</i> | — | D/2 : (80/10-15) : U/(E) : I/O |
| <i>Main characteristics:</i> | Contain double-stranded DNA, mol. wt. about 80×10^6 , G+C content 35 to 59%. Particles are bacilliform, about 40 to 70 nm \times 250 to 400 nm with an outer membrane and an inner electron-dense core. The particles can be occluded in a crystalline protein inclusion body, which may be rounded and contain only one or, rarely, two particles (granulosis, viruses, subgroup B), or be polyhedral and contain many particles (polyhedrosis viruses, subgroup A). Ether- and heat-labile. These viruses have been so far isolated from Lepidoptera Hymenoptera and Diptera, occasionally, Neuroptera. | | |

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|------------------------------|--|----------------------|-----------------------------|
| <i>Genus</i> | <i>Herpes virus group</i> | <i>Herpesvirus</i> | (D/2 : 54-92/7 : S/S : V/0) |
| <i>Type species</i> | <i>Herpes simplex virus</i> | — | D/2 : 68/7 : S/S : V/0 |
| <i>Main characteristics:</i> | Contain double-stranded DNA, mol. wt. 54 to 92×10^6 . G+C content 57 to 74%. Virus particle about 100 to 150 nm diameter. Capsid icosahedral with 162 hollow capsomeres, 100 nm diameter, with a lipid-containing membrane and therefore sensitive to lipid solvents. | | |

The DNA is about 7% of the particle weight. BD (CsCl) 1.27 to 1.29 g/cm³. Development begins in the nucleus and is completed by the addition of protein membranes as the virus passes into the cytoplasm. Intranuclear inclusion bodies formed. Separate subgroups have been proposed for viruses which are not readily separable from the cell and for the cytomegaloviruses. There are, however, such gradations in the properties that neither proposal is practical.

| | | |
|-----------------------|--|------------------|
| <i>Other members:</i> | Herpesvirus simiae (B virus) | : */*: */*: S/S: |
| | Herpesvirus of Cercopithecus (perhaps 2) | V/0 |

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|------------------------------|---|----------------------|------------------------------------|
| <i>Genus</i> | <i>Adenovirus</i> group | <i>Adenovirus</i> | (D/2 : 20-25/12-14 : S/S : V/0) |
| <i>Type species</i> | <i>Adenovirus type I</i> | — | D/2 : */13 : S/S : V/0 |
| <i>Main characteristics:</i> | Contain double-stranded DNA, mol. wt. 20 to 25 × 10 ⁶ . G+C content 48 to 57%. Isometric naked particle with icosahedra symmetry, 70 to 90 nm diameter, with 252 capsomeres, each 7 nm diameter. Vertex capsomeres are antigenically distinct from the others and carry a filamentous projection. Ether-resistant, BD (RbCl) 1.34 g/cm ³ , 795 s. Assembly in nucleus. Some haemagglutinate cells of various species. Many are oncogenic in certain conditions. A common antigen shared by all mammalian strains differs from the corresponding antigen of avian strains. | | |

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|-------------------------|---|----------------------|-------------------------------|
| <i>Group</i> | <i>Cauliflower</i> <i>mosaic virus</i> group | — | (D/2 : 5/15 : S/S : S/Apl) |
| <i>Type member</i> | <i>Cauliflower</i> <i>mosaic virus</i> (cabbage S isolate) | — | D/2 : 5/15 : S/S : S/Ap |

Main characteristics: Contain double-stranded DNA, mol. wt. about 5 × 10⁶, G+C 43%. Isometric particles about 50 nm diameter, about 220 s, no accessory particle, thermal inactivation point : 75 to 80°C, longevity in sap : a few days, concentration in sap probably 10 to 100 mg/l, mosaic and mottlesymptoms, narrow host range, aphid vectors, persist a few hours in feeding aphids, mechanically transmissible, serological relationship between members.

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|-------------------------------|---|----------------------|---|
| <i>Genus</i> | <i>Polyomavirus</i> group (Papova- virus subgroup B) | <i>Polyamavirus</i> | (D/2 : 3/7-13 : S/S : V/0) |
| <i>Type species</i> | <i>Polyomavirus</i> | — | D/2 : 3.4/13 : S/S : V/0 |
| <i>Main characteristics :</i> | Contain double-stranded circular DNA, G+C ratio 41 to 49%, mol. wt. about 3×10^6 . Particles 43 nm diameter. Capsid naked, composed of 72 capsomeres in a skew arrangement. 240 s. BD (CsCl) 1.34 g/cm ³ . Assembly in the nucleus. Inapparent infections in most hosts. Some are oncogenic under certain circumstances. Several haemagglutinate by reacting with neuraminidase sensitive receptors. Ether-resistant, acid-stable, heat-stable. | | |
| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
| <i>Genus</i> | <i>Reovirus group</i> | <i>Reovirus</i> | (R/2 : $\Sigma 15/15$: S/S : V/0, D1) |
| <i>Type species</i> | <i>Reovirus type 1</i> | — | R/2 : $\Sigma 15/15$: S/S : V/0 |
| <i>Main characteristics:</i> | Contain 10 to 20% double-stranded RNA in several pieces, total mol. wt. about 15×10^6 . G+C content 42 to 44%. Isometric capsid with icosahedral symmetry, usually naked but pseudomembranes, probably of host origin, are described. BD (CsCl) 1.31 to 1.38 g/cm ³ 630 s. Capsid diameter 75 to 80 nm. Two layered capsids. Resist lipid solvents. Virus synthesis and maturation in cytoplasm with formation of inclusions sometimes containing virus particles in crystalline arrays. | | |
| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
| <i>Genus</i> | <i>Leukosis virus</i> complex | <i>Leukovirus</i> | (R/1 : 10-13/1-2 : S/* : V/0) |
| <i>Type species</i> | <i>Rous sarcoma</i> <i>virus</i> | — | R/1 : 10/2 : S/* : V/0 |
| <i>Main characteristics:</i> | Contain single-stranded RNA, mol. wt. about 10 to 12×10^6 . G 25 to 30, A 19 to 26, C 22 to 27, U 22 to 29. Enveloped particles about 100 nm diameter. Nucleo-capsid of unknown symmetry (possibly helical). Ether-sensitive, heat-labile. RNA-dependent DNA polymerase reported in virus particle. Maturation by budding from cytoplasmic membranes. Sensitive to actinomycin D. Some are oncogenic. Four subgroups. Members within groups A and B share antigens. | | |

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|------------------------------|--|-----------------------|---------------------------|
| <i>Genus</i> | <i>Myxovirus (influenza group)</i> | <i>Orthomyxovirus</i> | (R/1 : 2-4/1 : S/E : V/0) |
| <i>Type species</i> | <i>Influenza virus (Ao/ws/33)</i> | — | R/1 : (2-4)/1 : S/E : V/0 |
| <i>Main characteristics:</i> | Contain about 1% single-stranded RNA, mol.wt. about 2 to 4×10 ⁶ , G 17 to 21 : A 20 to 23 : C 23 to 27 : U 31 to 36. Helical capsid 9 to 10 nm, probably in 6 separate pieces. Enveloped particles 90 to 120 nm spherical or elongated with characteristic surface projections. Haemagglutinate by virtue of neuraminidase-sensitive receptors. Possess Nucleocapsids form in nucleus, maturation by budding. Actinomycin D-sensitive. Genetic recombination common. Antigenic variation frequent. Antigenic crossing within subtypes. Three discrete antigenic types distinguished by specificity of RNP antigen with no cross-reaction. | | |

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|------------------------------|--|------------------------------------|-----------------------|
| <i>Group</i> | <i>Tobacco mosaic virus group</i> | <i>Tobamovirus (proposed name)</i> | (R/1:2/5:E/E : S/0) |
| <i>Type member</i> | <i>Tobacco mosaic virus (Common =Ul strain)</i> | — | R/1 : 2/5 : E/E : S/0 |
| <i>Main characteristics:</i> | Contain about 5% single-stranded RNA, mol. wt. about 2×10 ⁶ , straight tubular particles about 190s, helical symmetry with pitch 2.3 nm, protein subunits of mol. wt. about 17,500, infective particles about 300 nm long, thermal inactivation point: over 90°C, longevity in sap : years, concentration in sap often over 1 mg/ml, symptoms mostly mosaics and mottles, spread mechanically, efficient natural vectors not known, serological relationship between members. | | |

| <i>Taxonomic status</i> | <i>Current name</i> | <i>Approved name</i> | <i>Cryptogram</i> |
|-------------------------|---|----------------------|---------------------------|
| <i>Group</i> | <i>Turnip yellow mosaic virus group</i> | — | (R/1 : 2/36 : S/S : S/Cl) |
| <i>Type member</i> | <i>Turnip yellow mosaic virus (Cambridge isolate)</i> | — | R/1 : 1.9/37 : S/S : S/Cl |

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- Main characteristics:** Contain about 36% single-stranded RNA, mol. wt. about 2×10^6 , G 17 : A 22 : C 40 : U 21 (subgroup a), isometric particles of about 110 s and 30 nm diameter with 180 protein subunits in obvious pentamer-hexamer clusters, mol. wt. of subunits about 20,000, accessory particles of 50 s are empty protein shells. Thermal inactivation point usually 70 to 90°C, longevity in sap: a few weeks, concentration in sap often 50 to 500 mg/l, mosaic and mottle symptoms; narrow host range, beetle vectors, persist several days in beetles, mechanically transmissible, serological relationships between viruses within each of two subgroups.
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Chapter Eight

HOST-VIRION INTERRELATIONSHIPS

The extreme parasitic nature of the viruses is unique in nature. This sort of a relationship with living organisms must have evolved and diversified along with the evolution of life on this planet. As we understand the phenomenon now, it has several dimensions. Attempts of virus particles to invade and successfully inhabit different kinds of host cells is one of them. The other is the response offered by the latter. Consequential modifications undergone by the virions, successful infection on the part of the virion, influence of the events on the host etc., are all facets of this complex relationship. It should also be remembered that the total impact of such a relationship is quite distinctive and varies widely from one system to another, though, the operative framework, as outlined above, remains the same. We shall here consider some of these aspects in the light of our current understanding of the situation.

INFLUENCE OF VIRUS ON HOST ORGANISM

Viral infection can have many effects on an organism, ranging from mere symptomatic infection to acute disease conditions or even to induction of malignant growth and death. Generally, on successful infection, the host cell may be influenced in three principal ways. These are the causations of (1) no apparent change (also called *Latency* or *Carrier* state), (2) Cytopathic effects leading to death (3) Hyperplasia (enlargement in size) alone or sometimes followed by death of the cell. The basic mechanisms leading to these effects are apparently different.

Inapparent Infections

One of the important facets of viral studies concerns the existence of infections which do not show any visible or clinical symptoms. Such cases are referred to as *Inapparent* or *Sub-clinical* infections. The phenomenon is called *Latency* (not to be confused with latent period of the replication cycle), and the virus involved, since it cannot be detected, is known as *Occult* virus. According to K. M. Smith (1962),

there are four distinct categories of latency.

In the first category come those viruses which cause clear symptoms initially, during the time of infection. These, however, disappear soon but may recur at intervals as a consequence of some stimulus. The common example of this type of viruses is herpes simplex virus infecting man.

Secondly, there are examples where viruses causing a disease are harboured by the host even after its recovery. However, the disease symptoms do not recur. The virus may be passed on to the next generation of the host, though its influence on the latter may get less and less. Several plant viruses, like tomato black ring virus, fall in this category. Hosts harbouring such latent viruses could be detected by inoculating their sap into a healthy host, which would occasionally show the disease symptoms.

The third type of latency involves those viruses which apparently never cause a disease in their original hosts. These cannot even be induced to do so. In these cases the hosts act as mere reservoirs. Their detection is also difficult. Detection becomes possible only when other potential hosts are infected, accidentally or otherwise, by the sap of the original host. Latent infections of *Cuscuta* and a few weeds are the examples of this type.

The fourth category represents a fundamentally different condition. In this case the host appears to be free of any detectable virus until the latter are activated or stimulated to become suddenly active and lyse the cells of the host. These commonly occur in insects and in lysogenic bacteria.

Latent viruses are said to exist in host cells as 'chronic infection' or 'endosymbiotic infection.' These descriptions envisage the existence of a steady state equilibrated condition between the host and the virion. Another important feature of such a relationship is that it is maintained without apparently taxing the host cell overmuch. We may say, therefore, that some sort of 'peaceful coexistence' or 'balance of power' is operative in such cases.

Another term used to characterise the state of latency is *masking*. It was proposed by Bennet and was defined as a condition in which a virus is actively present in a plant without causing obvious effects, regardless of the causes of such absence of obvious effects (Bennet 1959). He had also suggested that the non-appearance of disease symptoms in some cases could be explained as due to low or reduced virus concentration.

More recently another interesting hypothesis has been proposed by Huebner and Torado (1969) seeking to explain the nature of latent

infections. They based their observation specifically on the role of RNA-C-type viruses in inducing tumors in vertebrates. Accordingly, most or all vertebrates contain C-type virus genomes which remain latent or repressed under normal conditions. Change in normal conditions may lead to derepression of the viral genes leading to obvious effects like tumorous growth.

Cytopathic Effects

Cytopathic effects mainly consist in the causation of morphological changes in the host cells. Sometimes these alterations may even lead to cell death. There may be various types of such changes, depending upon the host-virus system. For instance, we may cite the lytic effects of bacteriophages and cyanophages on the host bacterial and cyanophycean cells respectively.

The effects of some animal viruses on their host cells are slightly different. Adenovirus infected cells round up and aggregate in clusters before their eventual death. Cells affected by polio-virus round up and shrink, eventually getting totally destroyed. In hosts infected by plant viruses, cytopathic effects lead to partial or total chlorosis (loss of chlorophyll) of the chlorophyllous parts. For example, the various mosaic diseases of cucurbitaceous and solanaceous plants show such symptoms (Plate XII). Apart from chlorosis, another impact of viral infection of plants is the unequal growth of cell wall leading to all sorts of curvature of different organs, particularly, leaves. Often such curvatures accompany the chlorotic symptoms. Shrinkage and clustering of cells is another common influence, which mostly lead to cell death (Plate XIII). Table 8.1, summarises the cytopathic effects of some viruses on their respective hosts.

The basic explanations of the various cytopathic effects are still to be fully obtained and appreciated. For one thing, these are of many types, associated with different host virion systems. Therefore, the bases of such influences might not be the same in all the cases. In bacteriophage, cyanophage and some mammalian virus infected cells, depression of essential metabolic processes occurs. Primarily, the synthesis of host cell DNA is interfered with, though syntheses of RNA and proteins are not affected. On the other hand, RNA viruses such as TMV (tobacco mosaic virus), are known to inhibit cellular syntheses of RNA and proteins without in any way affecting DNA synthesis by the host cells.

Another reason behind the adverse influences on host cells is the forced release of the hydrolytic enzymes (lysozymes) from the organelles that contain them. This results in partial or total cell destruction.

TABLE 8.1. CYTOPATHIC EFFECTS OF SOME VIRAL INFECTIONS

| <i>Virus</i> | <i>Host</i> | <i>Cytopathic Effects</i> | <i>Symptoms</i> |
|------------------------------|-------------------------|--|---|
| Bacteriophage T ₄ | <i>Escherichia Coli</i> | Lysis due to release of hydrolytic enzymes | Plaque formation |
| Influenza | Man Mice | Secretion of toxins causing cell necrosis | Fever; Body ache |
| Rabies | Dog Cat | Development of inclusion bodies (Negri bodies) | Infection of central nervous system leading to convulsions |
| Tobacco Mosaic | Tobacco plant | Chlorophyll synthesis hampered; unequal cell wall growth | Chlorosis; Mottling |
| Vaccinia | Man | Alterations of the cell membrane | Aggregation and deformation of cells leading to external 'pock' marks |

With a few other viruses, particularly those having lipid membranes (lipoviruses), like the myxoviruses, infection leads to disintegration of host cell membrane into fragments which are utilised in the formation of the membrane coverings of the virus particles. In such cases, even the antigenic properties of the membrane fragments are known to alter to be acceptable to the viruses. In cells infected with herpes virus, the intervening membranes of contiguous cells dissolve resulting into the formation of giant cells.

In the case of plants infected with viruses, various physiological disorders are known to take place. In a review Diener (1963) has concluded that the physiological derangements most commonly associated with plant virus infection are the following:

- (a) increased respiration rate,
- (b) decreased photosynthetic activity,
- (c) decreased activity of growth regulators,
- (d) accumulation of soluble nitrogen components such as amides, and
- (e) increased activity of polyphenyl oxidase activity and accumulation of oxidized phenols.

All these changes taken together lead to various cytopathic effects.

The above mentioned influences are usually linked with the process of viral replication. However, there are a few effects which occur without or sometimes partial formation of progeny viruses. A high concentration of virions or of viral coat proteins is known to initiate cytopathic effects that are collectively referred to as 'toxic' effects. Such effects, more often than not, lead to rapid cell death, probably by

irreversibly destroying cell permeability. Symptoms associated with such human diseases as influenza, like high fever and body ache, are due to these toxic effects. Similar impact is also associated with limited viral synthesis or even in the absence of replication. For instance, viral pneumonia is known to be caused by inoculating large doses of Newcastle disease virus, without detectable multiplication of the virus.

A kind of effect associated with viral infections is not cytopathic in the earlier sense of the term but leads to alterations in the morphology of the host cells. These could be due to (1) developments of inclusion bodies (2) induction of chromosomal aberrations and (3) cell transformations.

Inclusion Bodies

Intracellular inclusion bodies are observed in quite a large number of host-virion systems. These could develop as a consequence to the aggregation of virions or their components at a particular site in the cell at the time of their formation. Eventually these appear to develop as crystalline structures upto a certain size after which lysis occurs resulting in the liberation of the particles. These structures could develop in the cytoplasm, as in the cases of cells infected with rabies virus forming *Negri* bodies or Mung bean mosaic virus (Plate XIV). Cytoplasmic crystals are also formed in insect cells infected by polyhedroses and granuloses viruses.

Sometimes, the crystals may be formed in the nucleus. As for example, in the case of adenovirus infection of mammalian hosts, or the crystals formed in the nuclei of insect cells by nuclear polyhedroses viruses. Occasionally, these crystals are known to appear in both the nucleus and the cytoplasm of the infected cells, as in measles. Most of these inclusion bodies are quite conspicuous and serve as diagnostic characters. Often the diseases are named according to the nature of these inclusion bodies.

In certain cases conspicuous bodies develop at the site of earlier viral infections that might have taken place. However, these bodies do not seem to contain detective virus particles or their components. The development of eosinophilic bodies in cells infected simplex virus is a case in point. Similar other instances include the development of basophilic entities in cells infected by yellow fever virus.

Chromosomal Aberrations

Cells in culture, when infected by a variety of viruses, may show chromosomal aberrations, like 'breaks' and 'constrictions.' Often such aberrations appear in the cells of organisms infected by herpes virus

and by polyoma virus. Some of these aberrations are general in nature but a few are highly characteristics. For instance, the 'breaks' induced by herpes virus occur only at certain sites of two specific chromosomes. More significantly, the progeny of the host cells continue to show such aberrations.

Cell Transformations

Some viral infections lead to alterations in the morphology of the infected cells. These viruses may or may not be tumour producing. However, infections by both result into morphological changes which more often than not cause enlargement and disfigurement of the cells (Plate XV). Cells growing in suspension cultures are more prone to such alterations. Transformation can also be recognised by (1) presence of intranudeal T-antigens, (2) loss of contact inhibition, (3) ability to cause tumours when inoculated into suitable hosts, and (4) new surface antigens (*see* Takemoto *et al.* 1971).

The mechanism of such transformations are not easily understood. However, evidences indicate that loss of control of the host cell on cellular metabolic functions, particularly those involving the informational macromolecules leads to such malignancies. Alternatively, it has been suggested that the control over the host cell metabolism is not altogether lost. Rather, viral genes take over the control from the host cell genome and bring about the changes. However, it is not clear as to what purpose. Notable examples of cell transformations are the various sarcomas caused by Rous sarcoma virus (RSV) and Simian virus 40 (SV 40).

Hyperplasia or Tumorous Growth

Hyperplasia or tumorous growth associated with plant or animal tissues is understood to be a mass of abnormal, disorganized, independently growing tissue which is without any physiological function but shows abnormal metabolic activity. A mass of such new growth is called a *Neoplasm* (neo=new; plasm= substance). Such growths could be *benign* i.e. localised to a particular tissue and growing very slowly. In contrast to these, some forms of neoplasm are *malignant* which proliferate extensively by invading adjacent tissues and cells and inducing them to do likewise. In higher animals neoplastic cells are often transported via the body fluids, like blood and lymph to distant areas.

Different types of tissues are known to be induced into tumorous growths or cancers. Agents causing cancerous growth are called *Oncogenic* (Onkos=mass of swelling). Viruses causing cancers are,

therefore, referred to as *oncogenic* viruses. One of the first such viruses to be discovered was the chicken sarcoma virus reported by Peyton Rous in 1911. Some twenty years later, Shope found that fibroma of rabbit was associated with viruses (Shope 1933). A few years later Bittner (1936) established that viruses were associated with mammary cancer of mice. Subsequently Gross (1951) demonstrated that leucoemia in mice was caused by a virus. He also showed that a second virus now known as polyoma virus, was associated with the leucoemia causing preparation and was capable of causing a variety of different types of tumours in rats and mice. Thus as early as in 1936 all the three types of oncogenic growths found in vertebrates was found to be associated with viruses. Since then many more oncogenic viruses have been discovered. Table 8.2 lists some of these along with their characteristics. Leucoemia, Sarcoma and Carcinoma are the cancerous diseases associated with blood/lymph, connective and epithelial tissue systems respectively.

TABLE 8.2. SOME ONCOGENIC VIRUSES AND THEIR CHARACTERISTICS

| <i>Virus</i> | <i>Host</i> | <i>Disease</i> | <i>Special Features</i> |
|-------------------|----------------------------|----------------|---|
| Adeno | Hamster | Carcinoma | DNA viruses; cause transformation of epithelial cells RNA C-type virus; Endonuclease, Cause blood cell transformation |
| Murine Leucoemias | Mice; Rat | Leucoemia | |
| Papilloma | Dogs; Cattle; Rabbit | Carcinoma | |
| Polyoma | Mice; Rat | Sarcoma | DNA virus; benign warts developed; epithelial cells transformed; in rabbit the disease is mosquito transmitted and becomes malignant |
| Rous Sarcoma | Chicken | Sarcoma | |
| Simian Virus 40 | Rabbits Pigs | Sarcoma | RNA virus; growth of subcutaneous tissues and pock-like tumors induced; transforms fibroblast cells |
| Wound Tumor | Legumes | Tumor | DNA virus; causes transformation of cells in culture Viruses with double stranded RNA; disorganised growth of root cortical tissue |

Viruses are known to induce tumorous growth in tissue cultures as well. Their infections often lead to the development of characteristic cell alterations, designated as cell transformations. When such transformed cells are implanted onto healthy tissues, cancerous growths result.

Often the cells transformed subsequent to the initial infection do not contain any virus particle at all. It is presumed that the mere contact of a previously transformed cell leads to such a change. Possibly the contact itself leads to some alterations in the cell membrane.

Conversely, when cancerous cells are grown in tissue culture, they behave as transformed cells. In general, the transformed cells acquire new traits with relation to cellular activity, antigenic property, chromosome morphology and overall growth characteristics. Since the behaviour of the transformed tissue culture cells are remarkably similar to that of the naturally occurring malignant cells, the former are used to study the mechanism involved in virus induced neoplastic growth.

General Characteristics of Oncogenic Viruses

Oncogenic viruses occur in both RNA and DNA containing viral groups, while both these types exhibit the capacity to induce tumorous growth in their respective hosts, there nevertheless exist between them a number of differences. It is also becoming increasingly clear that the mechanisms of induction by these two groups of oncogenic viruses are also different.

Important DNA containing oncogenic viruses include such diverse groups as Pox viruses (Vaccinia), Adenoviruses, Papoviruses, Herpesviruses and Parvoviruses. The influence of these virus groups in inducing tumorous growth are usually not very severe. In most cases only benign tumours are induced. Cell transformation is often associated with these tumorous growths.

Oncogenic RNA viruses, however, are quite restricted in their distribution. They form a rather homogeneous group of spherical viruses of about 110 nm diameter and with a RNA molecule having a molecular weight of about 11×10^6 dalton. This group is known as *Leukovirus* and includes such viruses as Rous Sarcome Virus (RSV), Murine Mammary Tumour Virus (MTV), Murine Leukaemia Virus (MLV) and Avian Leukosis Virus (ALV). Apart from leukoviruses, certain types of reoviruses are known to be oncogenic. Wound tumour virus, which causes tumour in sweet clover, is a double stranded RNA virus. More recently another RNA virus, called tobacco tumor virus has been associated with crown gall in tobacco callus cells (Misra and Nienhans 1976).

Although the leukoviruses exhibit an overall homogeneity, there are a few minor differences in morphological details. Accordingly, these viruses were grouped into a few subgroups. One of these subgroups are capable of inducing solid tumours, for example the murine leuka-

mia virus, rous sarcoma virus and mouse sarcoma virus. Most of these viruses with the notable example of mouse mammary tumour virus (MTV) are capable of maturing by a well defined budding process and are designated as *C*-type viruses (Bernhard 1960). The mouse mammary tumour virus (MTV) differs from other type-*C* viruses in having more numerous surface projection and a different alignment in the nucleoid and is designated as type *B* virus. Further, unlike DNA containing tumour inducing viruses, *C*-type RNA viruses are known to be naturally associated with leukaemia or solid tumour in chicken and mice and possibly in a host of other organisms (see Chattopadhyay *et al.* 1974).

Mechanism

Early researches carried on the mechanism of viral carcinogenesis revealed several important features. First, that viral oncogenesis appeared to be a manifestation of cellular modifications consequent to the introduction of viral genome into the host cell. It was also now known that Simian Virus 40 induced tumour cells contain a new virus specific antigen called specific Tumour (*T*) antigen, which continued to appear in the progeny of the originally infected cell, indicating the viral genes which produced this tumour inducing antigen must have become incorporated into the genomes of the transformed host cells. This phenomenon was considered, therefore, a type of infective heredity, analogous to infective heredity associated with lysogenic conversion. It had also been demonstrated that malignant or transformed cells contained viral nucleic acids which however, are functionally incomplete, *i.e.*, they never operated/leading to viral multiplication. Also, it had been shown that malignant or transformed *c* cells may not harbour any virus particles at all (see Gross 1961; Dmochowski 1959).

The other feature which throws some light on the mechanism of viral carcinogenesis relates to the modified or altered metabolic pattern of the diseased cells. It appears that the normal control mechanisms governing cell metabolism become totally non-functional. However, this does not mean that cellular metabolic processes become disorganised. Rather a new behaviour pattern, apparently controlled by viral genes, emerges, leading to the development of new traits. These include (1) alternations in the nature of the cell surface, which acquires new antigenic properties, and (2) total modification of cellular metabolism, gearing it to production of specific tumour (*T*) antigen (see Dulbecco 1967).

Several other aspects of the problem remained unresolved. These were obviously linked to the general understanding of the mechanism

of viral method of infection, replication etc. In recent years increasing studies are being carried out to understand the mechanism as operative at a more specific level. The mechanism of induction by C-type RNA viruses has been investigated in particular (see Temin-1971; Temin and Baltimore 1972).

Researches have revealed that oncogenic RNA viruses are found naturally associated with transformed or even cancerous cells but do not lyse them. This situation is unique and definitely indicates a special type of relationship between the host and the virion, where both can exist and multiply together. It has been suggested that the viral genes may not be fully expressed, possibly as a result of the host virion interaction. However, under certain conditions such as exposure to changing environmental condition or the age of the host cell a more complete expression results and leads to such phenomena as cell transformation and neoplastic growth (Howatson 1971).

More recent investigations on the role of murine leukemia virus, a C-type oncogenic RNA virus, in causing neoplastic growth in various mouse strains indicate that the C-type viral genomes are integrated into the host chromosome (Lowy *et al.* 1976). These integrated viral genomes, therefore, pass on vertically from one generation to the next and do not normally infect other individuals horizontally. However, certain conditions, like artificial induction with X-rays or treatment with base analogues, lead to large scale production of viral nucleic acids in the host cells. This is correlated with oncogenic effects on the host cell itself. (Lowy *et al.* 1974, Chattopadhyay *et al.* 1974, Nanson and Stephenson 1977). It has been also suggested by these authors that the degree of oncogenic or transformation effects are possibly related to the number of copies of the viral genome which are integrated with the host genomes and also to the extent the integrated viral genomes are expressed.

Two interesting questions that come up in this respect are (i) how the viral genome, which is RNA, gets integrated with the host genome which is DNA and (ii) why does the viral genome remain non-expressed while in association with the host genome.

The answer to the first question was suggested and confirmed by Temin, Baltimore and others who showed that the viral RNA genome produces a single stranded DNA complementary to itself with the help of a specific enzyme RNA directed DNA polymerase (reverse transcriptase) (see Temin 1971). This complementary DNA strand then gets associated with host genome, possibly in a manner similar to the genome of coliphage lambda while the latter associates with *Escherichia coli* K₁₂ strains.

The second question also is still to be answered fully. It is however reasonable to accept the suggestion (see Howatson 1971) that the integrated viral genome remains repressed ordinarily. This, however, does not prevent it from replicating along with the host genome and thus getting transferred hereditarily. This would indicate that while the capacity for replicating itself, even in an integrated state, is not hampered, its capacity to act through the normal channels of *m*-RNA production (transcription) and enzyme protein production (translation) is curbed. Some studies indicate that the repression might be at the level of transcription (Lowy *et al.* 1976). Under certain undefinable condition the viral genome gets derepressed and produces more RNA particles thus increasing the quantity of virus particles endogenously and ultimately leading to the oncogenic effects. The mechanism of cell transformation and oncogenesis by DNA viruses is not very clear. For one thing these viruses, with the exception of the pox viruses and Simian Virus 40, often have a very different kind of effect on the host cell which sometimes leads to its lysis. Further, these viruses are rarely, if at all, associated naturally with their hosts. In most cases their oncogenic effects have been demonstrated by artificial inoculation of huge doses of virus particles in tissue culture.

The role of a few DNA containing oncogenic viruses like Simian Virus 40 (SV40), Polyomavirus and Adenovirus have been investigated in more details. These viruses are all capable of transforming host cells and of creating hyperplasia. There are evidences to indicate that several copies of the DNA of these viruses get associated with the host cell genome in a manner analogous to the behaviour of temperate phages. Subsequently when cell transformation occurs, the transformed cells are found to contain virus specific *m*-RNA molecules. Virus specific antigen, namely antigen *T* (tumour) and TST (tumor specific transplantaion) are also found in transformed or tumour cells indicating virus activity in these cells. Absence of new virus particles in the cells transformed with adenovirus or Polyomavirus is attributed to incomplete transcription of the viral genes possibly due to specific protein repressors (see Howatson 1967).

More recent studies (see Weissbach *et al.* 1976, Martin *et al.* 1974; Martin 1976) on the molecular basis of the mechanism of the effect of SV40 on mammalian cells indicate that:

- (1) SV40 has two types of effect on the host cell; it either lyses it or remains abortive.
- (2) Cell transformation is associated with abortive infection.
- (3) Both the effects are associated with *T* antigen.
- (4) However, the *T*-antigen isolated from lytic infection was found

to be different from the one found associated with transformed cells and was probably needed for successful replication of SV40 particle.

(5) The T-antigen associated with lytic infection was the initiator of DNA synthesis.

(6) The T-antigen associated with cell transformation acts as some sort of regulator in the host cells.

ROLE OF VIRUSES IN HUMAN CANCER

One aspect of viral carcinogenesis that is undergoing extensive investigation these days concerns their possible role in human cancer. The two important possible human tumour viruses are (1) C-type viruses and (2) Herpes virus. Polyoma and related virus are also regarded as potential carcinogenic agents.

There are few early reports which indicate that C-type RNA viruses could be associated with human leukemic tissue (Dmochowski *et al.* 1967). The association of C-type RNA viruses with chicken, mice, cats are beyond doubt. That they are associated with leukemic primate cells is also on record (Benveniste *et al.* 1974). Any positive proof that human leukemic cells contain them is still to be reported. However there few reports that leukemia specific DNA sequence have been isolated from human cells as well (Baxt *et al.* 1973; Mondal and Motashaw 1976).

Herpes virus or herpes like viruses have been associated with Bukitt lymphoma, a cancer type reported from Africa (Epstein *et al.* 1965). Another disease, naso-pharyngeal carcinoma is reported to be associated with herpes viruses (Old *et al.* 1966). But whether these virus are really carcinogenic in these cells are still to be found. Also, evidences indicate that herpes virus II may be associated with human cervical cancer.

The forms of hyperplasia discussed above have a common feature. In these cases the infected cells are never killed. However, a type of hyperplasia is sometimes associated with cell death, apparently due to extremely rapid viral multiplication leading to exhaustion of the host cell resources. Such instances are to be observed in chorioallantoic membranes of chick infected by pox viruses. Characteristic pock marks appear due to c-cell death.

RESPONSE OF HOST CELLS TO VIRAL INFECTION

In the previous paragraphs we have seen how a successful viral attack can and does influence the behaviour of host cells or tissues. However,

not all infections of a particular host organism are successful. Prospective hosts are often indifferent to viral infection; they are just not infected at all. At other times they are able to resist or circumvent, partially or totally, the influence of the invading virions. All these things happen primarily because of certain built-in genetic mechanisms in the host cells that prevent or modify an attack from being successful. In general, these mechanisms are manifest in four principal phenomena, namely, 'host specificity,' 'resistance,' 'interference' and 'immunological responses of the host.'

Host Specificity

An important feature of viral multiplication is host specificity. As the term suggests, infection of a suitable host is essential if the progeny is to develop. In natural conditions, all viruses are broadly speaking, 'restricted' and will infect only a certain type of organisms. For instance, bacterial viruses infect only bacteria. Similarly, cyanophages and plant viruses will infect blue-green algae and higher plants respectively but not the fishes. In the same way, fish viruses will not infect mammalian hosts. However, there are certain viruses which specifically infect dual or even multiple hosts belonging to widely different taxa. For instance, vector mediated plant viruses infect their insect vectors as well.

Within a broad group of organisms, however, the specificity becomes somewhat lax. For instance, rabies virus is a mammalian virus and is capable of infecting a large number of mammals. Similarly, tobacco mosaic virus can infect an array of species belonging to the solanaceae. There are instances, however, where several viruses are known to infect only a particular host species. For example, smallpox, measles and polio viruses are capable of infecting only human beings.

It is evident, therefore, that a successful infection is dependent upon an undefined special relationship that exists between the host and the virion. It is not known whether the basis of such relationship is the same with all types of host-virion systems. The mechanism as related to the bacteriophage—bacterial system is known to involve a specific reciprocal adsorption between the bacterial surface and the phage tail fibres. As discussed earlier, such specific adsorption is based on the antigenetic properties of the two entities. Whether this behaviour is entirely genetically controlled or not is not fully understood. However, a few reports claim that under experimental conditions, the specificity of infection could be modified. This would indicate that physiology of the cells has an important role to play.

Resistance

A cell may not allow the invading virus to infect it successfully. Such host cells are generally referred to as 'resistant' types with relation to the invading viruses. The ability on the part of the host cell to prevent infection is termed *resistance*. This phenomenon becomes operative only when invasion is attempted and not otherwise. Lack of this trait on the part of a cell makes it non-resistant or *susceptible* to that invading virion. Resistance is, therefore, opposite of all that susceptibility connotes. Since the latter is a positive phenomenon vis-a-vis infection, its proper understanding is more likely to give us a better insight into the former.

Cell susceptibility, be it a bacterial cell or one belonging to plant or animal tissues, is dependent upon and is determined by the early stages in host-virion interactions. The crucial stages are, therefore, the attachment of the virions to the host cell surface and the subsequent events leading to release and penetration of their nucleic acid cores. With animal viruses and bacteriophages, and probably also with plant viruses, a successful infection must be preceded by a successful attachment or adsorption of the virion to the host cell surface. This is dependent upon the presence of specific receptor sites on the host cell surface as well as the corresponding sites for adsorption on the virion. Absence of the required specific sites would render the prospective host cell resistant to the virus in question. The exact mechanism behind such specific interaction between the host and the virion is not fully understood. However, antigenic attractions between the two are obviously involved.

The phenomenon of susceptibility and resistance to viruses reversible. A change could be induced at both genetical and physiological levels. A cell species ordinarily susceptible may undergo genetic mutation and be converted into a resistant type. This could be due to a change in the nature of the receptor sites. Hybridization between a resistant variety and a susceptible one may lead to the development of recombinations of either of the types. Physiological factors such as heat, *pH* status, presence or absence of metallic ions or of alien enzymes can influence the nature of the receptor site on the cell surface of a susceptible strain and convert it into a resistant type. Conversely, a resistant cell type, when grown *in vitro*, may become susceptible. Age of the tissue or the organism is also an important factor; for instance, poliomyelitis particles infect new born babies or young children but not the comparatively grown up individuals.

Interference

Sometimes, infection fails to occur because adsorption to specific sites is interfered with. The term *interference* is associated with all such phenomena. It has been shown that a majority of interfering processes are mediated through the agency of viruses. When virion of more than one type infect the small cell, each may multiply undisturbed. But in certain cases multiplication of one type may be totally or partially inhibited because of the concurrent multiplication of other types. Obviously, such inhibitions are virus induced and hence are referred to as *Viral Interferences*.

One of the first such phenomenon to be studied involved the Ring spot disease of tobacco caused by the virus with the same name. On initial infection, typical ring shaped lesions appear on the aerial parts of the host plant. These subsequently regress though the virions continue to be in host cells. However, subsequent fresh infection fails to produce any symptom whatsoever thereby indicating some sort of interference with the multiplication of the new entrant. Such observations were also found in case of animal viruses.

In 1957 the study of viral interference took a new turn when Isaacs and Lindemann discovered that interference in the multiplication of influenza virus particles was mediated by a substance produced in the infected cell itself. Since then similar reports with other virions have also been reported. These substances were subsequently given the generic name *Interferons*.

Investigations with isolated and purified interferons have revealed that these substances are glyco-proteinaceous in nature and are resistant to acidic pH conditions. These are moderately thermostable, being destroyed at temperatures higher than 50°C. These are not virus specific rather are host cell specific. In other words, the same virion will produce different types of interferons in different host cell types. These generally differ in their molecular weights. These have molecular weights which vary from one host virion system to another and the range may be as large as 15,000—100,000. They also differ in their antigenic properties. Further, when infected into uninfected susceptible cells, these induce the latter to become resistant. Further a given interferon inhibits viruses most effectively in the cells of the strains or species in which it was produced. The basis for such specificity is unknown.

The mechanism of production of interferons is not clearly understood. It has been noted that the production in host cell occurs regardless of whether the virus is infective or not. In other words its mere presence would induce the formation of interferons. Further the

amount of interferon produced appear to be inversely proportional to the rapidity of multiplication. A virus which is most effective in infecting a particular host (a virulent type) induces least interferon production. Conversely, an unsuccessful infection is concurrent with the production of larger quantity of the substance. Recent researches indicate that the formation of interferons involves the host cell DNA. These are induced and directed to transcribe specific messenger RNA molecules which in their turn are utilised in the synthesis of the required proteins. The viral nucleic acids present in the host cells are known to play some role in this induction. Recent studies indicate that the inducer molecule could be a double stranded RNA molecule. However, why and how does such a molecule gets induced is not clear as yet.

Interferon action is explained in two ways. In the first place, they are known to interfere with the syntheses of viral proteins. This is naturally mediated through the prevention of the activity of viral messenger RNAs, presumably by interfering with its alignment to the host cell ribosomes. How this is exactly achieved is still not clear. Another mode of action is directed towards reducing the chances of the host cell getting infected. It, therefore, has a protective role. Since interferons produced in a given cell can be absorbed by a wide range of host cells, at least partial protection to the susceptible strains can be ensured easily. This, therefore, has an important therapeutic role. Recent reports, that adsorption of reo virus particles to the host cell, its penetration and uncoating are prevented in interferon treated host cells, confirm this idea (Lengyel *et al.* 1976). In fact in some cases effective therapeutic role has been claimed (*see* Ho and Armstrong 1975). Ability of interferons to simultaneously inhibit cell multiplication of cell growth are also on record (Knight 1976). It has been shown that the human fibroblast interferon, a glycoprotein can perform both the functions.

Apart from interferon production, interference can be achieved through competition between different viruses infecting the same host cell. This has been shown to occur when two bacteriophages attack the same cell but at different times. Almost always, the phage infecting earlier dominates and slows down the replication by the other. In case of some animal viruses, adsorption by the second virus is prevented. With still others the process of replication might be blocked at different intermediary stages.

Immunological Responses of the Host

It is well known that vertebrates and some invertebrates possess a

built-in natural defence mechanism. This capacity is due to the ability of these organisms to produce a protein when a certain kind of substance, which is normally foreign to its tissues, enters into them. The foreign substance is known as the *Antigen*. The protein produced is called the *Antibody*. These are made up of gammaglobulin type of protein molecules and are referred to as immunoglobulins. Five different kinds of immunoglobulins are known to occur in human systems. These are extremely specific in characteristics in that a particular kind of antigen (either a protein or a carbohydrate mostly) induces the production of a particular type of antibody alone. Antibodies combine chemically with its inducer antigens. If the antigen happens to be some disease causing organism, like viruses, they also induce the production of antibodies. These antibodies then specifically combine with the virus particles themselves. Such combination totally blocks the disease causing ability of the viruses. The viruses are said to have been neutralized. Herein lies the importance of this phenomenon as a defence mechanism for the host.

Antibodies present in serum (blood minus the corpuscles and clotting constituents) and extracellular body fluids provide the main protection against viral infection. Antibodies may be present as such or may be introduced into the system artificially (vaccination or artificial immunization). The degree of protection is directly related to the amount of antibodies present. In both the cases they are able to neutralize the infecting virus particles.

The mechanism of neutralization is not fully understood. Several aspects of the mechanism are, however, clear. It is recognized that neutralization does not require saturation of the virion with antibody molecules. Rather, a single antibody molecules can effectively do so. What is of fundamental importance is that the antibody molecules should combine with the infective virion in such a way as to prevent the virion from infecting the prospective host cell. To be able to infect, a virion must have an intimate contact with its host cell surface. If one antibody molecule is able to bring about this prevention, then it effectively neutralizes the virion. If it cannot do so then more such molecules will be needed.

Apart from protection neutralizing antibody molecules also play an important role in recovery from viral infections. The reason behind such a phenomenon is not clearly understood. Another related aspect concerns persistence of antibodies in the body system. This could be due to persistence of antibody production once the stimulation to produce them has been received by the host cell. It may be also due to presence of inapparent infections or may be due to repeated infection

in a short duration of time.

Host Induced Modification

A different type of response of the host results in the modification of the infecting virion itself. We may call them host induced modification. Sometimes, replication of a virus in certain host types may alter its infective properties. Such changes occur due to reasons that are basically associated with the response of the host to infection, and hence, are referred to as the host induced changes. These alterations were originally noted in phages infecting strains of the bacterium *F. coli*. It was observed that some of these phages can infect a particular host strain only but not the others, and are known as the *restricted* types. Occasionally, these types of viruses are known to acquire the capacity to infect hitherto resistant host strains and are said to have become *unrestricted*. Similarly, unrestricted phages may become restricted. In fact reports are there which claim that the progeny of a phage strain fail to infect the bacterial strain infected by the parent. Since such alterations appear only after the phage undergoes at least one multiplication cycle, they are undoubtedly host induced.

Experimental evidences confirming the existence of such a phenomenon mostly come from the studies on *F. coli* strains infected by coliphage T_2 . Isotopic investigations have revealed that some of these strains release preventive nucleases (nucleic acid destroying enzymes) on being infected by phage virions. These enzymes destroy the nucleic acid of the virion thus preventing further spread of infection. The bacterial strains, therefore, behave as resistant strains. Bacteria susceptible to the said phages normally do not possess the aforementioned nucleases.

Such hydrolysis of the viral nucleic acid in the resistant hosts could be blocked by its previous glucosylation (attachment with a glucose residue), a step which renders it possible for the phage nucleic acid to make the nucleases infructuous thus helping the virion to proceed on with the infection of the host cell. Glucosylation could be achieved if the phages infect a susceptible bacterial strain which is both a non-producer of the nucleases as well as a possessor of the glucosylating enzymes. Progeny of such virions would all have glucosylated nucleic acid. When these infect a resistant strain, the nucleases would have no effect at all. The infection would be complete and the restricted phage would behave as an unrestricted one.

This sort of situation is known to be operative in certain strains of *E. coli*. which are originally resistant to the coliphage T_2 . However, when these restricted phages are initially made to infect strains of

Shigella which are susceptible but possess the glucosylating enzymes, their progeny starts behaving unrestrictedly and infects the hitherto resistant bacterial strains. Figure 8.1 depicts graphically a hypothetical conversion from restricted to unrestricted phage.

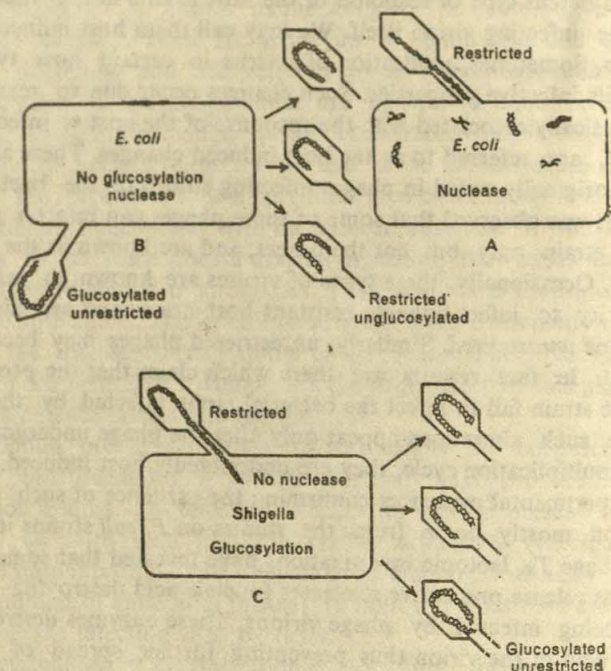


FIG. 8.1. Host induced modification of a non-infective virion into an infective one. Certain strains of *E. coli* possess nucleases (nucleic acid hydrolyzing enzymes.) These destroy the phase nucleic acid when it enters these bacterial cells. These bacteria are thus resistant to the phages concerned. However, if the phage nucleic acid is previously glucosylated (attached to a glucose molecule) then it can escape destruction even if the host cell has nucleases. Glucosylation can be achieved when a phage infects a susceptible strain (hence without nucleases) which has the necessary glucosylating enzyme (A). The progeny of this phage will all have glucosylated nucleic acid core and will be able to infect a resistant strain. Thus, a phage which was earlier non-infectious becomes modified and behaves as an infections one. However, when such changed phages infect bacterial strains without glucosylating enzymes their progeny are all incapable of infecting a resistant host (B and C).

Apart from glucosylation of the viral nucleic acid, there are other means by which the restricted forms may be converted into the unrestricted ones. With the bacteriophage *P₁* infecting certain strains of *E. coli* such conversions are brought about by methylation of the DNA.

of the restricted types. Other host induced modifications include morphological alterations of the virus. For instance, host cells are known to induce changes in the morphology of the particles by converting them up with portion of its (host's) own membrane, as in case of certain animal viruses.

Such changes are not heritable since the characteristics are primarily dependent upon the necessary enzymes of the host cell. Thus a transformed virion would be able to produce unrestricted progeny only if it infects a bacterium carrying the required enzyme system. There are other features as well which distinguish such host induced changes from the truly genetical ones. For one thing, these occur in a single cycle of modification in a modifying host. Also, in such cases all the cells are abruptly altered and not a few as would be expected with genetical changes. Again, such a change could be reversed by a single passage through a host lacking in the necessary enzymes.

We have tried to project an idea about the various principal facets of host-virion inter-relationships. The complex nature of the events leading to successful invasion of a particular host by a virion and the various means adopted by the host cell to prevent or circumvent the virion from getting established, point towards the extremely intricate nature of viral parasitism. These events also suggest the possibility of viral forms existing in diverse host taxa, probably at different levels of adaptation. Evidences of the host cell harbouring two viral forms, one in a latent state and the other being infectious, are numerous. Conversely, many plant diseases are known to be caused as a result of mixed infections by two virions, often acting complementary to each other. All these things indicate that viral parasitism is in a state of dynamic flux and is probably still undergoing radical alterations. Perhaps, in the proper elucidation of the various aspects of the phenomenon would lead us to the answer to the riddle of biological status of these agents.

VIRAL DISEASES—THEIR TRANSMISSION

PREVENTION AND CONTROL

Previous chapters have shown us how viruses, for whom parasitism is obligatory, multiply in the host cells and varyingly influence the host cells. We have also noted the interactions between the host and the infecting virion, with not infrequently adverse influence on the former. These effects could collectively be referred to as the diseases caused by viruses. All the different organisms in whose cells virus multiplication might occur, are in a position to get diseased on being infected by viruses. Viral diseases are of common occurrence in plants, insects and animals. Some of these are listed, and a few discussed, elsewhere (*see* Chapter Ten). At present, it is not our aim to discuss the etiologies of these diseases in their entirety; we shall mainly consider the general symptomatology, means of transmission and the prevention and control of these diseases.

SYMPTOMS OF VIRAL DISEASES

Viral infections could be categorised into three basic groups, namely, (i) Localised; (ii) Systemic or Disseminated; and (iii) Inapparent infections. Localised infections include those cases where viral multiplication and damage occurs in cells at and near the point of entry. The spread of virus particles is extended to cells in direct contact with the primary infected cells. On the other hand, systemic infections rapidly spread to regions quite distant from the site of initial infections and sooner or later the entire organism becomes infested with virus particles. Inapparent infections, as mentioned earlier (*see* Chapter Eight), are not immediately detectable.

Symptoms may be defined as the external manifestations of these infections with the help of the disease can be diagnosed. For obvious reasons only localised and systemic infections are expressed as symptoms. These symptoms may appear at the site of infection or at certain regions irrespective of their distance from the site of infection.

There is no specificity in the nature of symptoms vis-a-vis infection in the sense that widely different symptoms can be caused by the same virus infecting different hosts. Conversely, the same symptom may be caused by different viruses. Also, symptoms of the same infection may vary in the same plant with changes in age, nutritional status and growth conditions. On the whole, therefore, symptoms may be regarded as reactive expressions of viral infections having considerable applied importance in recognising and thereafter, fighting a disease.

Symptoms of Viral Diseases of Plants

Plant diseases caused by viruses are mostly systemic in nature. Although the entire plant harbours the virus particles, only a few organs exhibit the symptoms. Generally, leaves show the most characteristics of these symptoms. In some cases, shoots, floral organs or even roots show the symptoms. However, the most striking effect is the general reduction in growth, vigour and cropping power of the plant.

Of the usual symptoms associated with these diseases, the most common is *Chlorosis* or change in the colour of leaves and other green parts. Light green or yellow patches of various shade and shapes or sizes appear in most chlorotic leaves. In some these patches may form characteristic *Mosaics* (Plate XII). With certain others there may not be any mosaic of patches, rather a general chlorosis or *Mottling* type of symptom may be exhibited (Plate XIII). Apart from these two types of chlorosis, *Vein-clearing* and *Vein-banding* are also common and usually precede mosaic and mottle formation. Vein-clearing involves onset of chlorosis of veins and veinlets leaving the rest of the leaf green. The situation is reversed with vein-banding. Sometimes vein malformations are simultaneously noted. Apart from chlorosis, other types of colour changes are also deserved. For example, the bluish-green colour of leaves associated with blue dwarf of oats.

Besides changes in colour, the foliage may show various types of growth malformations, mainly as a consequence to differential growth rates at the different growing regions or surfaces. There may be curling of leaves, inwards or outwards, resette formation and various non-descript distortions. Enations or outgrowths may also appear on the leaves, often leading to reduction of leaf growth.

There may be growth distortions at other plant parts as well. For example, the shoot portion of the plant may show swelling or occasional tumorous growths may develop. The stem may show retarded growth or *Stunting*. Twisted or knotty growth of many virus infected cucurbitaceous plants is a common feature. Tumors may also be

formed in roots as in case of rumex or Sweet clover plants.

Often prolonged infection leads to complete destruction or necrosis of the infected spots. Infected parts such as leaves appear to be randomly perforated. Sometimes necrosis may occur in the vascular and cortical elements of infected leaves and stems. In a few cases terminal buds or the entire top may become necrotic. Phloem tissues become necrotic in some cases.

Of the other symptoms, there may be changes in colour of petals or breaking, induction of sterility, insipidity of fruits, wilting of plants *et cetera*. Table 9.1 summarises the symptoms associated with some well known viral diseases of plants.

TABLE 9.1. GENERAL SYMPTOMS OF SOME PLANT VIRAL DISEASES

| <i>Disease</i> | <i>Symptom</i> |
|---------------------------|--|
| Apple mosaic | Yellowish patches on the leaf which coalesce later leading to necrosis. |
| Banana bunch top | Leaves become short and narrow get bunched together at the top of the plant. |
| Bhindi yellow vein mosaic | Vein clearing followed by chlorosis, fruits dwarf, malformed. |
| Citrus quick decline | Lack of growth of new flushes, yellowing of leaves, Limbs die back from tip downwards. |
| Papaya leaf curl | Leaves become curled and develop mosaic. |
| Potato leaf roll | Characteristic rolling of leaves which become leathery. |
| Pumpkin mosaic | Mosaics leading to mottling. |
| Rice Tungro | Leaves turn yellow from tip downwards, plants show stunting bearing empty grains. |
| Tobacco mosaic | Chlorosis leading to mosaic; leaf curl and distortions; mottling. |
| Tobacco leaf curl | Curling and distortion of leaves. |
| Tomato bunch top | Stunted growth leading to bushy appearance. |
| Tulip mosaic (break) | Variegation of flower petals. |
| Wheat mosaic streak | Linear mosaics which meet together with spread of infection and become dry. |

Internal Symptoms

There are certain characteristics of virus infected plants which could be termed 'internal symptoms.' For example, necrosis in phloem elements in diseases like potato leaf roll and sugar-beet curly top. However, the most prevalent of internal symptoms are the 'inclusion' bodies, which have been often used as diagnostic features.

In general, two types of inclusion bodies have been recorded. These are (i) the crystalline and (ii) the amorphous types. The latter are also

known as the *X* bodies. Both the types may be present together in the cells of the same infected plant part. Again, they may be nuclear or cytoplasmic, present together or separately.

Crystals, whether cytoplasmic or intranuclear have reproducible regular features, sufficient for considering them of diagnostic importance as for example, the inclusion bodies associated with mosaic of tobacco, bean yellow mosaic and tomato bushy stunt (Plate XIV).

X-bodies are not entirely made of virus particles. In certain cases, as in mosaic of tobacco, virus particles are observed to be adsorbed on the surface of a core portion. In some other cases, as in cells of *Datura stramonium* virus infected with tobacco etch virus, the *X*-bodies consist of a peripheral lipid zone and an internal matrix containing virus particles.

Apart from these two categories, there are various peculiar kinds of inclusion associated with virus infection. For example unbranched tubules observed in cells of Cowpea infected with Cowpea mosaic virus.

Symptoms Associated with Viral Diseases of Animals and Human Beings

The clinical symptoms associated with animal and human disease are, in general, identical in themselves and are less specific than those seen with the diseases of plants. The most common symptoms are fever, general body ache and chill as in case of influenza. External and internal necrotic lesions appear in some cases of poxes. Paralysis of the limbs is the most striking feature of poliomyelitis. Rashes on the skin are associated with diseases like measles. A few viruses like Herpes

TABLE 9.2. GENERAL SYMPTOMS OF SOME VIRAL DISEASES OF ANIMALS

| Disease | Symptom |
|----------------------------------|--|
| Chicken pox | External lesions, bodyache, fever. |
| Encephalitis | High Fever, bodyache, Headache. |
| Hepatitis | Liver malfunction, Jaundice. |
| Influenza | Chill, fever, generalised ache of the limbs. |
| Kyasanur Forest Disease | Fever, Typhoid like symptoms. |
| Poliomyelitis | Paralysis of the limbs, fever. |
| Rabies | Hydrophobia; Difficulty in Swallowing, increased muscle tone leading to spasmodic contraction. |
| Small pox | Characteristic external and internal lesions, Necrosis. |
| Common cold and related diseases | Fever, nasopharyngeal secretions. |
| Gastro-intestinal diseases | Fever, occasional rash, digestive disorders. |

virus are known to induce tumorous growths. Gastro-intestinal disorders are also known to occur in certain cases. Liver malfunction results in case of viral hepatitis (Table 9.2).

Viral diseases of animals and human beings are usually focussed on certain 'Target Organs.' Wherever the initial infections might be, ultimately the infection spreads to these organs. Central nervous system (CNS) and the respiratory tracts are two such target organs. Systemic infections, moreover, may spread to different regions of the organism.

Physiological Changes Leading to Clinical Symptoms

It should be clearly borne in mind that the aforementioned clinical symptoms are the generalised external manifestations of the alterations at cytological and biochemical levels as a result of viral infections. We have already discussed the broad features of these modifications (*see* Chapter Eight). Suffice it to say here that these effects are the results of host-virion interactions favouring the invading virions. The nature and the extent of these modifications vary from infection type to infection type. In most cases specific metabolic patterns of the host are known to be affected.

TRANSMISSION OF VIRAL DISEASES

The modes of spread of viruses, and consequently the diseases spread by them, are many. To some extent, these means are dependent upon the nature of the host organism. In general, two principal categories of means of transmission are noted to be operative. These are (1) those mediated through an intermediary organism, called the *Vector* and (2) the ones transmitted without the intervention of any vec-

TABLE 9.3. MODES OF TRANSMISSION NOT MEDIATED BY VECTORS

| <i>Disease</i> | <i>Mode</i> |
|------------------|--------------------------------|
| Bean Mosaic | Seed |
| Lettuce mosaic | Seed |
| Potato leaf curl | Contact |
| Tobacco mosaic | Man implements |
| Tobacco necrosis | Soil |
| Tomato bunch top | Bulbs, rhizomes |
| Hepatitis | Water |
| Influenza | Nasal & oral discharge |
| Myxoma of rabbit | Dust, contact |
| Poliomyelitis | Faeces, sputum |
| Rabies | Bites of infected dog, fox |
| Small pox | Close contact, scales, sputum. |

tors. Though such a categorisation, on the fact of it, seems to be arbitrary, there are several fundamental differences in the nature of

operation of the two types. For one thing, mediation by vectors means its involvement with the virion in a complicated relationship which might often be biological. On the other hand, modes that do not involve vectors are largely mechanical.

Transmission of Viral Diseases not Mediated by Vectors

A list of some viral diseases transmitted through various means not involving vectors is given in Table 9.3. We shall briefly consider here a few of them in some detail.

Air Borne Transmission

This is probably the most important mode of transmission of animal viruses. Viral diseases involving respiratory infection are mainly transmitted by this means. Droplets expelled from the mouth or nose of infected persons contain a large number of virus particles. Usually these are attracted by gravity and reach soil ultimately. However, small droplets, 0.1 mm or less in diameter become smaller still due to rapid evaporation and before reaching the surface, they float in air for many hours or even days, as 'droplet nuclei.' These 'nuclei' can easily be inhaled and lead to further infection. Even after such droplets reach some objects, like furniture, bed linen or dust particles, they remain infectious. The various influenza viruses are transmitted in this fashion.

Among the other important air transmitted viruses are the small-pox and the poliomyelitis viruses infecting human beings and the virus causing Newcastle disease of fowl. Dried epidermal scales from small-pox pustules are a ready source of infection. Similarly, airborne fragments of feather or excretions from parrots affected by psittacosis, if inhaled, can cause infections in human beings. Plant Viruses are not known to be transmitted through air.

Transmission Through Contact

Transmission through contact, direct or indirect, is the main method of transmission with many plant viruses. This is particularly true of diseases of crop plants. A good example of such transmission is that of potato virus-*X* which spreads in the field of contact between both roots and crown of infected and healthy individuals. Contact between aerial parts is most common in strong breeze. The same virus may be infected by getting carried on the person or the implements of farm workers.

Of the various viruses affecting animals and human beings, the foot and mouth disease virus is spread by contact. Even the milk of cattle infected by this virus is infectious, the infectivity being retained even

when the milk gets dried. With fowl pox disease direct contagion is most frequent. Infection is facilitated by the presence of wounds and abrasions on the host surface.

Hereditary or Vertical Transmission

This is one of the important modes of transmission of insect viruses. These are most commonly disseminated through the eggs of the infected parents. Earlier, a controversy raged as to whether the virions were passed through the infected embryo or were merely carried on the shell. It is now abundantly clear that the embryos in such cases are indeed infected. In fact in some cases the young larvae are known to die of the disease even before emerging out of the shells. High incidence of inapparent infection in insects is another point indicating built in source of infection. Large insect populations may carry such latent infection generation. The infection becomes detectable only after these are stimulated into activity.

Hereditary transmission is not uncommon with animal viruses. Such diseases as small pox, chicken pox and rift valley fever are known to be transmitted through the placenta of the mother. An important case of transmission of a virus from parent to offspring is that of breast cancer of mice. It has been established that such passage occurs solely through the mother's milk. Murine leucemia virus (MLV) is also known to be vertically transmitted in mice.

Transmission of viral diseases of plants through the agency of various propagating agents is of normal occurrence. Transmission through seeds is known to occur in lettuce mosaic virus caused diseases. Transmission of viral disease through the means of vegetative propagation is of most common occurrence. All plants viruses which are systemic in their hosts are carried through such propagating organs as tubers, bulbs, rhizomes, cuttings and buds. Most of the diseases of potato, of plants grown of bulbs and of raspberries are passed on by this method. Ratoon stunt disease of sugar cane is transmitted in this manner from one generation to another.

Some Other Modes of Transmission

Of the other modes, transmission followed by ingestion of contaminated food by insects is interesting. The food is generally a plant. Previous contamination of the food plant may be direct, either by excretion of the infected larvae or due to the presence of their disintegrated body on the plant surface. It could also be indirect, being transmitted by wind, bird or any other agent. When such infected plants are eaten by the insects, the viruses are ingested. Once inside,

the tissue disintegrate and the aggregated virus particles are liberated. The free particles are released in the gut, ultimately come out and are carried further. Apart from plants, dead bodies of already infected insects may be the contaminated food.

Usually insect viruses are transmitted in this manner *e.g.*, the polyhedroses and granuloses. These exist inside the host as well defined crystals protected by proteinaceous membrane. Since they appear as aggregations, their dispersal is relatively easy. After the death of the carrier insects and the particles get free, further transmission takes place. Often these particles may get carried *en masse* to fresh plant surfaces and contaminate them. Sometimes, insects parasitic on other insects may also act as carriers of virus particles.

Another important mode of transmission without involving vectors relates to the plant viruses causing soil borne diseases. These are generally carried through the agency of soil micro-organisms. Often direct mechanical contact between roots and virus is sufficient for the spread of a disease, as with tobacco necrosis. Wound or abrasion on the root surface facilitates such transmission. Naturally grafted plants like *Cascuta* are also conducive to transmission of viral diseases. Water borne transmission is also known to occur. In fact, in tropical countries like India, occurrence of such infectious disease as infectious hepatitis (jaundice) is spread through contaminated water.

Artificial Inoculation

Artificial transmission of viruses has been practised from very early days of virus pathology. The idea was to see if virus could be transmitted under laboratory conditions thus facilitating collection of experimental data relating to organisms (mainly plants) otherwise resistant to particular virus infections. Two procedures of artificial transmission are generally prevalent, namely, (1) mechanical inoculation; and (2) grafting.

Mechanical inoculation. Mechanical inoculation of host individuals is achieved very simply. The sap or extract of the infected host is used as the inoculum. Crude extract could be used as the inoculum very conveniently, particularly if used fresh. Standard methods of extracting the sap have been developed. Sometimes, it is necessary to chemically treat the extracted sap in order to prevent its destruction, by oxidation. For example, saps of tomato spotted wilt has been found to remain stable only when treated with sodium sulphite (Na_2SO_3). Phosphate buffer of low molacity (0.03 M) has also been found useful in this regard.

The leaves of plants to be inoculated are dusted lightly with some

abrasive materials such as carborandum powder. Then a piece of muslin or even the forefinger is dipped into the mocolum and the leaves are rubbed very gently. Lastly, the excess mocolum is washed rapidly off the leaf surface. Sometimes stiff artists brush is used for applying the mocolum. In case of mass mocolation high pressive spraying has been successfully used.

Almost all the important plant virus diseases like tobacco mosaic, tobacco ring spot, tomato spotted wilt and mosaic of bean have been sub-transmitted artificially.

Grafting. Viruses that are systemic in their hosts can be transmitted by grafting between susceptible or compatible plants. In case of herbaceous plants, the leafy upper portions (*scion*) of one individual are suitably trimmed and inserted into the cut stem of another (*stock*). Usually the scion is virus infected rather than the stock. There may be several modifications of this procedure.

Tuber and bulbs grafting have also been practised successfully as in case of potato viruses and tulip viruses.

Sometimes when the two partners are mutually incompatible, i.e. (infection cannot be passed from one to the other e.g. cucumber mosaic virus from its natural host to *Nicotiana glutinosa*, transmission can still be achieved by bridging the two, as it were, by grafting using the stems of *Cuscuta* species.

Grafting has been found a successful means for transmission of such viruses as Alfalfa mosaic virus, potato virus Y, tomato spotted wilt virus, tobacco mosaic virus etc.

Vector Mediated Transmission

As mentioned earlier, many viral diseases are dependent upon other organisms for their transmission from one host to other potential hosts. The agents responsible for such transport are called *vectors*. Most, though not all, organisms acting as vectors are insects. Other arthropods such as mites also sometimes play an important role. A few nematodes and even fungi are known to act as vectors for virus diseases.

It should be emphasized here that it is obligatory on the part of the vectors to be able to feed on the host plants or animal in question. Another point to remember is that the relationship between vector and a virus it carries is seldom mechanical; more often than not, the vector acts as an intermediary host for the virus. In the following paragraphs we shall look into the various aspects of vector mediated transmissions.

Vectors and Plant Viruses

The vectors of plant viruses mostly belong to the insect order Hemiptera. A great majority of them belong to the sub-group Aphidae and one of them *Myzus persicae* acts as vector for more than 50 viruses. A few are transmitted through members belonging to the Coleoptera, Thysanoptera and Lepidoptera as well. Arthropods apart from insects also act as vectors. Table 9.4 lists some of the important viral diseases of plants and the vectors involved in their transmission.

The aforementioned insects get involved in viral transmission primarily owing to their food habit and their manner of uptake of the food. In this context the architecture of feeding parts and feeding habits assume considerable significance (Fig 9.1). Hemipteran insects (aphides) have sucking mouth parts and, therefore, feed by sucking the plant sap directly. The sap flows through the mouth parts carrying the virus particles. It has been recently suggested that the turgor pressure of the cell sap itself pushes the latter up the mouth parts and onto the mouth cavity. Saliva of the insect, which flows down the beak like mouth parts into the host cells, also plays an important role in upward movement of the sap, presumably by acting as a suitable medium.

The members of coleoptera (beetles) and lepidoptera (grass-hoppers) feed by biting at the food and ingesting it. These insects do not secrete saliva. Rather, they spread the disease by regurgitating the injected material, which in coming with contact of healthy susceptible host plants enables the virus to achieve fresh infection.

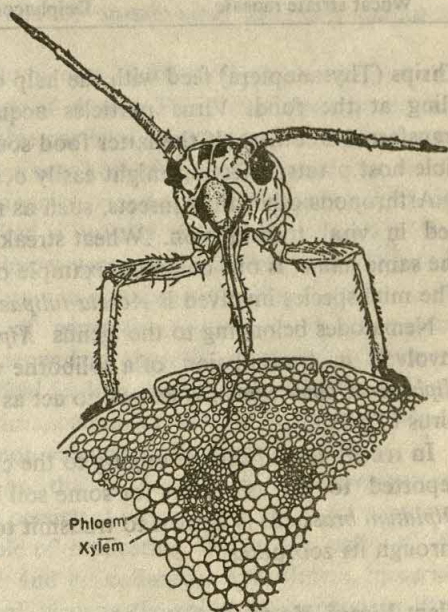


FIG. 9.1 Copy of a drawing by Busgen (1891) showing an aphid in feeding position with the stylets inserted in the phloem part of a vascular bundle of a stem of *Papaver collinum*. Virus particles present in infected phloem cells can easily move into the style along with the fluid that might be taken in. (After Esau, 1958).

TABLE 9.4. PLANT VIRUSES AND THEIR INSECT VECTORS

| <i>Virus</i> | <i>Vector</i> |
|----------------------|--|
| Bean mosaic | <i>Myzus persicae</i> (Aphid) |
| Citrus quick decline | <i>Myzus persicae</i> (Aphid) |
| Maize streak | <i>Balclutha Nibila</i> (leaf hopper) |
| Papaya mosaic | <i>Aphis gossypi</i> (Aphid) |
| Rice Tungro | <i>Nephotettix apicalis</i> |
| Tobacco leaf curl | <i>Bemisia tabacci</i> (white fly) |
| Potato leaf roll | <i>Myzus solanifolia</i> |
| Tomato spotted wilt | Thysanoptera (Flirios) |
| Wheat striate mosaic | <i>Delphacodes pellucida</i> (Pland hopper). |

Thrips (Thysanoptera) feed with the help of one mandible only, nibbling at the food. Virus particles acquired at one place thus gets transferred to others. If the latter food sources happen to be susceptible host plants, infection might easily occur.

Arthropods other than insects, such as mites, are sometimes involved in viral transmission. Wheat streak virus causing the disease of the same name, is one common example of mite transmitted viruses. The mite species involved is *Aceria tulipae* heifer.

Nematodes belonging to the genus *Xiphenema* are known to be involved in transmission of a soliborne virus diseases. For example, *Xiphenema americanum* is known to act as vector for tobacco ringspot virus (TRSV).

In recent years fungi belonging to the chytridiomycetes have been reported to act as vectors for some soil borne viruses. For example, *Olpidium brassicae* is known to transmit tobacco necrosis virus (TNV) through its zoospores.

Plant Virus—Vector Relationship

The relationship between plant viruses and their insect vectors has been a matter of considerable interest from the very early days of studies concerning insect mediated viral transmission. In general, these relationships can be divided into two categories, namely, one in which a clear biological association exists between the virus and its vector and the other where no such involvement occurs. Both the types may be linked to the various aforementioned vectors. However, occurrence of biological relationships are more prevalent. The two groups are widely different in their ranges as well as in their modes of operations. All these aspects could be fruitfully discussed with relation to the behaviour of an important insect vector of plant viruses, the aphids.

Aphids are known to be associated with transmission of viruses mechanically and also as partners in a well defined biological association. The mechanically transmitted viruses are sometimes referred to as being *non-persistent* since these are not retained by their vectors for long. In most instances, the capability of infection is lost by the vectors after one infection. All lose this capacity in due course. The ability may be reacquired on feeding afresh on infected plants. Even the shortest of feeding times enables the insects to acquire the faculty. However, longer feeding period ensures acquisition of larger number of particles and thus increases the chances of new infections. Bean yellow mosaic virus and pea enation mosaic virus are two examples of non-persistent types.

Viruses that are biologically involved with their insect vectors are known as the *persistent* type of viruses. The vectors associated with such viruses retain the infective particles for long periods and, therefore, are capable of infecting a new host after a considerable lapse of time. In most cases, the capacity once acquired becomes a life long faculty and one acquisition feed is usually enough to maintain the infective reservoir of the insect vectors. The amount of the feed or the duration of feeding is of no consequence with regard to the infection frequency of the vectors of persistent viruses. In other words, the capacity to infect is not dependent upon the amount or the duration of feeding; rather a critical feeding time is essential for acquiring infectivity. Potato leaf roll virus, cucumber mosaic virus and barley yellow dwarf virus are common examples of the persistent type.

Investigations carried out on the mechanism of the relationship between a vector and the persistent virus that it might be carrying, reveal that the virus is capable of replicating inside the cells of its aphid host. Dr K.M. Smith and his colleagues have shown, by serial transmission of potato leaf roll virus in the aphid *Myzus persicae* that even the latest host is capable of infecting susceptible plant. Further, they observed that the capacity to infect remained undiluted in the aphids at each level of transmission. These two observations clearly indicated that the virus had been multiplying in the aphids.

Persistent viruses can also be transmitted hereditically. It has been demonstrated by Black (1950) that clover club-leaf virus can be transmitted upto the twenty-first generation through the egg of the vector, a leaf hopper *Agalliopsis novae* Say. Each generation has been observed to retain the level of infectivity of the previous generation.

Another way of looking into the virus-aphid relationship, proposed by Kennedy *et al.* (1962), is being increasingly used in the literature. Accordingly, aphid transmitted virus are categorized into (i) stylet

borne; (ii) circulative; and (iii) propagative types.

Stylet borne viruses. These are those viruses which adhere to the insect stylets after penetration of virus in the infected plant. A virus of this type can be immediately picked up by the insect and can be transmitted to a susceptible healthy plant. However, with successive penetration of a number of healthy plants, the capacity to infect decreases and sometimes stops only after the first plant. Similarly deeper penetrations than 15 nm into the infected part have resulted into decreased efficiency.

Aphid mediated transfer of stylet borne viruses has been explained as a mechanical process during which the virus gets adsorbed on the aphid stylet. On subsequent penetration of susceptible individuals, these adhered viruses cause infection. Apparently, adherence of viruses to stylets depends upon such factors as degree of penetration, presence of virus particles in infected cells, duration of feeding, role of rostrum etc.

While the mechanical nature of the process is not doubted much, one question remains unsolved. This concerns the aphid specificity for different viruses. A specific aphid alone can transmit even a stylet borne virus. A mechanical process and biological specificity are normally mutually exclusive.

Potato virus Y, bean yellow mosaic virus and beet mosaic virus are three examples of stylet borne aphid transmitted viruses.

Circulative type. These viruses show a relationship with their aphid vectors which is somewhat more intimate than is the case with the stylet borne viruses. Circulative viruses are taken in during feeding but the insect acquires the power to infect only after a lapse of time. This is probably due to the time taken by the virus to pass through the gut into blood and back to the salivary gland. In some cases like pea enation mosaic virus (PEMV) these viruses are retained by the aphid in their gut before being passed on to the blood stream and onto the gut. However, no clear cut biological relationship (in the sense of virus propagation) is known to exist between these viruses and their vectors. But the relationship is definitely not as mechanical as obtaining with stylet borne viruses.

Potato leaf roll and pea evation mosaic viruses are too well known viruses of this type.

Propagative type. This type includes those viruses which show a definite biological association with their vectors, akin to that of the persistent types mentioned earlier. Serial transmission of leaf roll virus showed that infectivity is retained even up to the sixteenth generation in the aphid *Myzus persicae*. Also that the degree of virulence

is not diminished. This indicated that the virus has been definitely multiplying in the vector.

Biologically persistent relationship of propagative virus is even more better exemplified in case of virus causing dwarf disease of rice, the leaf hopper *Nephotettix apicalis* Motsch. It has been established that the virus is transmitted from the infected parent leafhopper to its offspring but only through the female parent. The offspring attains infectivity after a lapse of time only. However infectivity with the same degree of virulence is to be found even upto the twenty first generations. In fact the presence of microcrystals of the virus in the guts of the vector have been observed under the electron microscope.

The propagative viruses also depict a clear cut specificity for their vectors. The specific relationship may be manifested in a variety of ways, such as (1) among different strains (2) among various developmental stages, and (3) among different forms of one species.

Propagative virus (or the persistent type) are thus dual host viruses with adverse affects only on one of the hosts, the plant in this case.

ANIMAL VIRUSES AND THEIR VECTORS

Many of the viruses infecting animals and human beings are vector transmitted. Since the days when Walter Reed and his associates demonstrated the involvement of a certain type of mosquito in the transmission of yellow fever virus, about eighty different types of vector-virus associations have been established. Most of the vectors involved are arthropods, a majority of them insects. Table 9.5 lists a few of these.

TABLE 9.5. A FEW ANIMAL VIRUSES AND THEIR VECTORS

| Disease | Virus | Vector | Host |
|---------------------|------------------------------|-------------|--------|
| Equine Encephalitis | Equine Encephalitis Virus | Mosquito | Horse |
| Poliomyelitis | Polio Virus | Cockroaches | Mouse |
| Foot and Mouth | Foot and Mouth Disease Virus | Birds | Cattle |
| Scrub Typhus | Scrub Typhus Virus | Mite | Man |
| Yellow Fever | Yellow Fever Virus | Mosquito | Man |
| Dengue | Dengue Virus | Mosquito | Man |
| Myxomatosis | Myxomatosis Virus | Mosquito | Rabbit |

Like plant viruses, animal viruses are also either mechanically associated and transmitted or are involved biologically with their vectors.

Of the mechanically transmitted viruses, rabbit myxomatosis virus is a good example. This virus is transmitted by a mosquito called *flying pin*. This mosquito feeds on diseased rabbits and in the process its stylets get contaminated. When the same mosquito happens to feed on a new host, the latter gets infected. However, if in the meanwhile the stylets somehow get decontaminated, by way of the virus particles being rubbed off, the mosquito loses its capacity to infect any further. Another example of mechanically transmitted virus is the virus causing fowl-pox. This is also transmitted by mosquitoes. Some other arthropodes, like the mites, are also involved in mechanical transmission of viruses.

Transmission of such viruses as causing yellow fever and encephalitis, involves definite biological association with the respective insect vectors. In both these instances, the capacity to infect is acquired not immediately on feeding but after a lapse of time. It has been established that the viruses, after being taken in by their vectors, moves along the lumen of the gut, reaches a particular site and multiplies there.

Another important feature of vector transmitted animal viruses is that the effectiveness of infection depends upon the stability of vertebrate-virus-invertebrate cycle. This stability would require that on one hand the infection of the vertebrate host by the insect vector be precisely timed and, on the other, the virus particles be capable of replicating within the insect vector while the latter is not invading new vertebrate host. The former would ensure that the vector takes in mature ineffective particles and the latter would keep the reservoir of ineffective particles filled in till vector invades a fresh host.

One important aspect of vector mediated transmission is the question whether any harm reaches the vector because of its carrier role. In this regard, the influence, on their respective vectors, is not identical with animal and plant viruses. The former do not seem to cause any adverse effect on their vectors. However, plant viruses are known to be harmful. For instance, the jassids (leaf hoppers) carrying peach-curl virus have been established to have a shorter life span in comparison to the normal individuals. It has been demonstrated that the leaf hopper *Delphacodes pellucida*, carrying wheat striate mosaic virus, has forty per cent less progeny than those fed on healthy plants. Further investigations on these aspects are bound to reveal more of such negative influences.

When we consider vector mediated transmission as a whole, it becomes apparent that the entire operation, irrespective of its being mechanical or otherwise, is secondary to the feeding habit of the vec-

tors. Carriage and transmission of viruses is primarily because of the organism on which the vector feeds being already infected. The development of biological association is, obviously, a secondary development. In all probability, such associations are a manifestations of the successful adaptation of the virus to the new hosts; a stable host-virus-vector cycle thus becomes established. The ability to replicate within the vector is the most remarkable step towards establishment of such stability and with it the eventual successful transmission of the infective virions.

CONTROL OF VIRAL DISEASES

Virus caused diseases affecting human beings, animals or plants have to be challenged and if possible, conquered before they become disastrous in the extreme. Indeed, it would be the safest thing for all concerned if the diseases could be prevented from occurring at all. As the next best alternative the endeavour should be to eliminate diseases by suitable curative treatments. Failure to achieve either of these would obviously lead to adverse influences which might include even death of the host organism. Other effects such as temporary disablement, paralysis, distortion or disfiguring of external surfaces, loss in yield in case of diseases of economically important plants etc. are known to be associated with a large number of viral diseases of plants, animals and humans.

The methods aimed at controlling viral diseases thus could be categorized as (i) preventive; and (ii) therapeutic or curative procedures. The aim of the former is to ensure that the disease is prevented from getting established in the host. The latter processes aim at eliminating the disease after its existence in the host becomes quite evident.

Prevention of Viral Diseases

We had suggested a little earlier that prevention of viral diseases from occurring involves stopping the disease from establishing itself in the host. This stoppage could be achieved by

- (a) exclusion of the source of infection;
- (b) isolating the source of infection;
- (c) protecting the host from getting infected; and
- (d) making the hitherto susceptible host a resistant one.

These broad processes, if applied at the proper time and in appropriate manner, are sufficient to prevent a disease from getting established. We consider a viral disease like yellow fever, which is non-existent in a certain area, say, India. Now the best way of preventing this

disease from getting established in even a single individual in India would be the exclusion of the source of infection. In other words, any carrier of this disease must be prevented from entering India.

In case this precaution fails and a carrier does manage to get in, then its isolation or eradication or complete curing must be attempted. Isolation is achieved by quarantine procedures. Eradication should be possible where the carrier is dispensable such as a plant specimen; otherwise, as an alternative, it must first be freed of the potential disease causing agent.

Protection of a host organism from getting infected can be achieved by circumventing the process of virus infection itself. For example, destruction of specific disease transmitting organisms, the vectors can help to a large extent in preventing the spread of a disease. Protection can also be attained by making the host immune to possible future infection. Vaccination, a process which utilizes the built-in immune response system of vertebrates, is a protective measure, commonly used the world over.

Exclusion of the Source of Infection

This method aims at preventing entry of the source of infection into an area where it is naturally non-existent. The usual course adopted by most governments is the application of quarantine laws restricting the entry of infection. Duly attested health certificates have to be carried by persons moving from one country to another. In the absence of these documents she or he is compulsorily secluded for a period of time. The idea is to isolate the person who might be a potential carrier till the fact is ascertained either way. The duration of isolation may vary from one disease to another.

Similar laws applicable to plant and animal sources are also enforced. If the source is dispensable, like an impotent plant tissue or plant specimen then it is best to destroy it immediately.

Isolating the Source of Infection

Isolation of the source of infection prevents its further spread. It is a normal health practice to isolate a diseased person suffering from small pox. You might be aware that such persons are usually quarantined at hospitals situated away from densely populated localities. It is always better not to keep them at their homes particularly in a large city like Delhi.

Isolation and complete eradication of dispensable hosts is a common practice in preventing plant diseases from spreading. As soon as the first few plants in a field are detected to be infected, the safest

way to prevent the disease from spreading would be to destroy the infected plant individuals. Wild (natural) hosts are similarly eradicated.

Contact with airborne diseases, like influenza could be avoided by avoiding crowded places like theatre, cinema halls etc. Personal hygiene also saves a lot of botheration. Public spitting or even coughing should be avoided. Care should be taken while rubbing eyes with fingers as it might lead to viral conjunctivitis of eyes.

Protection of the Host from Getting Infected

The third method of prevention is to protect the host organism against any potential infection successfully leading to disease. Protection does not, therefore, necessarily mean that the host would not get infected; rather its ultimate aim would be to ensure, somehow, that the organism designed to be protected does not get diseased. However, means aimed at avoidance of infection are also considered to be protective in nature.

Immunization

The most widely practised method of preventing a disease from getting established is *immunization*. This method, however, is effective only in cases of disease of vertebrates. This method aims at making the host systems temporarily resistant to disease. Such induction of resistance is based upon the ability of the lymph cells of the host organism to produce special type of protein entities called antibodies in sufficient quantity to counteract the foreign invasion. The virus particles would act as antigen and the corresponding counteracting antibodies will be produced in the host.

Antibodies could be produced in the host system by introducing into the latter attenuated (weak) or previously inactivated virus particles as well. These would have their antigenic properties leading to antibody production in the host cell without, however, harming the host system. Afterwards, infection by virulent viral particles would be successfully counteracted by the already present antibodies. Introduction of dead or killed virus particles also lead to similar results. Recent studies indicate that neutralization of infective virus particles by the host antibody prevents release of nucleic acid from the particles. It should be remembered in this connection that in animal (and in plant) cells ingestion of virus particles is total, i.e., the complete particle is more or less absorbed by the host cell (pinocytosis).

The pioneering case of immunization was the successful vaccination against small-pox, introduced by Edward Jenner in 1796. He used a

weak strain of cowpox or *Vaccinia* for immunizing non-infected individuals. The name of the procedure is derived from the name of the virus. Although this first effective immunization against a viral disease (or any disease, for that matter) antedates the actual discovery of viruses, the real break-through in this field could occur only in recent years. Such diseases as polio, yellow fever, influenza and rabies have all been successfully prevented from getting established in their respective hosts. Table 9.6 lists some of the diseases against which immunization has been effective.

TABLE 9.6. VIRAL DISEASES IN WHICH IMMUNIZATION HAS BEEN SUCCESSFUL

| <i>L. sease</i> | <i>Immunogen</i> |
|--------------------------|-------------------------------------|
| Poliomyelitis | Formalin Inactivated; Attenuated |
| Rabies | Phenol Inactivated; Attenuated |
| Small Pox | Active but Attenuated |
| Yellow Fever | Active but Attenuated |
| Measles | Active but Attenuated |
| Influenza | Inactivated; Heat killed |
| Mumps | Inactivated; Heat killed |
| Adenovirus Infections | Inactivated |
| Encephalitis | Active but Attenuated |

The usual vaccination procedures may be regarded to be of three kinds depending upon the nature of immunogen in use. Accordingly, there are procedures utilizing (1) inactivated or killed virus, (2) active but weakened and live attenuated virus, or (3) large quantities of antibodies from a recovered individual as the immunogen. With all these procedures, the basic objective of inducing the host cells to produce antibodies that would protect them from eventual infection when it occurs, is well served.

However, the effectiveness of these three types of immunogen varies. This variation, again is disease oriented. The type of immunogen to be used against a particular infection is, by and large, determined by trial and error. In general, live but weakened viruses act as better immunogen in most of the cases. This is probably because the development of complete immunity is favoured by long persistence of the immunogen in the body. Also, the amount of antibodies produced by live viruses is likely to be more mainly because of the self-replicating of live viruses.

The mechanisms of interaction between the host cell antibody and the invading virion is too complex to be considered here in detail. Suffice it to say that antibody molecules completely neutralize the infecting virion, preventing it from becoming harmful. It has been demonstrated that total neutralization does not require saturation of the surface of the virion; rather, in some cases, a single antibody molecule can neutralize one infecting particle. It has been suggested that binding of the antibody molecules to the virus particles prevents the latter from releasing their nucleic acid into the cell, probably by preventing the latter from releasing their nucleic acid into the cell, probably by preventing the required intimate contact between the virus particle and the cell surface. It, however, does not interfere with the adsorption of the virus particle to the cell surface.

Immunization of Plants

Immunization procedures are hardly applicable to plants as they do not give rise to antibody molecules, obviously because of total lack of immune response mechanism. However, plants infected by a particular viral strain are known to become resistant to attack from virus of the same strain as well as from other strains of the same virus. This fact could be of practical utility only when and if it becomes possible to infect a field crop deliberately with a mild strain of some infectious virus, thus making the plants resistant to the infectious strains. These procedures, however, are still in experimental stages. Prevention of viral diseases of plants can be affected by selecting and cultivating naturally immune varieties. In recent years, development of genetically resistant varieties of crop plants, such as, mosaic resistant sugarcane and curly top resistant sugar beet have proved successful in preventing the occurrence of these diseases.

Eradication of Vector

Another way to protect the host and to prevent viral diseases from spreading would be to avoid or to eradicate the vectors before they are able to spread the infection. Destruction of insect vectors could be achieved by using suitable insecticides. However, care has to be taken to ensure that the vector population in a particular locality is totally obliterated, since a few surviving individuals would be enough to spread the disease. For this reason, earlier use of such insecticides as DDT and nicotine sulphate were not much successful.

In recent years, development and use of systemic insecticides have proved more effective, in controlling insect vectors, and consequently in the reduction of vector mediated disease incidence. For instance,

tomato leaf-curl has been controlled in India by applying Parathion, a powerful systemic insecticide. Insects could also be prevented from acting as vectors by adjusting the dates of sowing or planting so that the period of insect activity is avoided and the vector-virus-plant cycle is disturbed.

Breeding Disease Resistant Varieties

This method is potentially the most fool-proof of all which aims at preventing a disease. This aims at changing the basic nature of the host thereby altering its relationship with the invading virus. The host becomes virus-immune. This method, however, is a long term one, as its development needs time.

Some success in this regard has been achieved already. Several good varieties of mosaic resistant sugar-cane have been developed. These strains proved extremely successful in reducing the adverse effects of the mosaic disease of sugar-cane to a large extent. Similarly curly top disease of beet in the USA could be checked by introducing disease resistant strains of sugar-beet (*see* Smith 1974).

TREATMENT OF VIRAL DISEASES : PROHYLAXIS AND THERAPEUTICS

Various methods have been adopted from time to time to eradicate viral diseases after they have established themselves in the host. These methods can be categorised as (1) those which attempt at the eradication of chemical agents, and (2) those which attempt so by physical agents. Success with these procedures has been variable, but none has yet proved to be fool-proof. We shall consider here a few important ones of these.

Chemotherapy of Viral Diseases

Attempts have been made to use chemical agents, both as prophylactic and as therapeutic to control viral disease. The most useful chemotherapeutic agents against bacterial diseases, namely; the sulphonamides and the antibiotics, were tried initially but proved to be largely ineffective. These drugs were useful against bacteria because of their selective retarding effects on the bacterial metabolism without influencing much the host cell metabolism.

However, as viruses are dependent upon the host cell biosynthetic pattern for their physiology, such selective influence becomes extremely difficult to achieve in case of viral infection; any adverse effect of these drugs on the viral replicating process is likely also to affect the

host cells adversely. Another reason for the ineffectiveness of antibacterial agents is that the specific enzyme systems sensitive to these drugs are absent in or unrelated to the viral systems.

The only group of antibiotics known to possess anti-viral activity are the rifamycins. These are produced by various strains of *Streptomyces mediterraneae* and are reported to be capable of repressing RNA, dependent RNA and DNA synthesis. Therefore, these are claimed to be particularly effective against RNA viruses.

Despite all these limitations, the prospects of antiviral chemotherapy are not altogether negligible. It has been pointed out by many virologists that selective eradication of virus in host cells is related to the quantitative differences that might exist in the sensitivity of a component common to the requirements of both the virus and the host. For example, by using controlled dosages of chemical agents, nucleic acid metabolism of viruses could be selectively inhibited without interfering much with the nucleic acid metabolism of the host cell. Other processes such as synthesis of capsid monomers and their assembly could also be interfered with. Similarly, successful adsorption to the receptor sites on the cell surface could be prevented.

As of now, successful treatment with specific chemical agents is still to be perfected. Extensive researches carried out in different laboratories all over the world have brought out some effective experimental antiviral chemical agents. These are mainly base analogues, i.e., chemical analogues of purine and pyrimidine bases required for the synthesis of nucleic acids. Some of these analogues, or more precisely, their corresponding nucleotides, compete with their normal counterparts for incorporation into the newly synthesised DNA. The latter thus come to have the analogues and are functionally ineffective, though structurally stable. The progeny virus often has incomplete capsids and is non-infectious. Analogues like 5-fluorodeoxyuridine are known to inhibit the enzyme thymidilate synthetase, and thereby prevent DNA synthesis. Table 9.7 lists the commonly used antiviral base analogues (Figs 9.2 and 9.3) on page 231 and 232.

Other important chemical agents used in antiviral therapy are the substituted benzimidazole and guanidine derivatives. These have been found to be effective against RNA viruses. It has been shown that these inhibit the formation of virus induced RNA polymerase, thereby preventing or retarding RNA synthesis. Another compound, found to be effective against pox-viruses, is isatin beta-thiosemicarbazone. This is known to selectively inhibit the synthesis of late structural proteins, apparently by interfering with the function of messenger RNAs. Perhaps the most successful antiviral agent to be used clinically

TABLE 9.7. CHARACTERISTICS OF SOME ANTIVIRAL BASE ANALOGUES

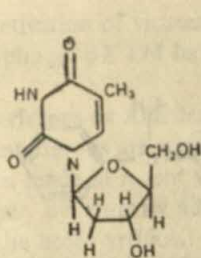
| Compound | Substitute For | Influence | Used Against |
|-----------------------------|----------------|---|----------------------------|
| 5-Fluorodeoxyuridine (FUDR) | Thymidine | Inhibits thymidilate synthetase; prevents DNA synthesis | Pox-viruses |
| 5-Bromodeoxyuridine (BUDR) | Thymidine | DNA synthesis; Interferes with maturation of particles | Pox-viruses Herpes simplex |
| 5-Iododeoxyuridine (IUDR) | Thymidine | DNA synthesis; Maturation of particles | Pox-viruses herpes simplex |
| Guanidine hydrochloride | Uridine | Inactivates RNA polymerase | Polioviruses Enteroviruses |
| 6-aza uracil | Uridine | Capsid formation | Tobacco mosaic virus |
| 2-Thio uracil | Uridine | Capsid formation | Turnip yellow mosaic |
| 8-Azo guanine | Uridine | Capsid formation | Peach yellow |

is *amantadine* and its derivative. These have been particularly found to be useful prophylactic agents against Influenza and para influenza. Characteristics of these compounds are given in Table 9.8.

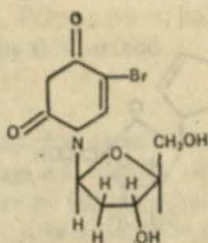
Although some of these compounds have been utilized with some success in certain cases, their overall utility has been, so far, restricted. The chemotherapeutic value of these drugs has been limited to superficial infections which can be exposed to the treatment without total involvement of the organism. Some of these drugs, like Isatin beta-thiosemicarbazone derivatives, have been found to be better prophylactic than therapeutic agents. It has been suggested that antiviral prophylactic drugs could be better utilized in the control of viral diseases, because therapeutic treatment could be applied only after the disease becomes extensive and the symptoms appear, and by then might become redundant or might adversely affect the host itself.

Chemo-Therapy of Viral Diseases of Plants

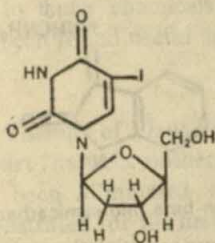
Most of the chemicals mentioned earlier have been used and experimented against animal viruses. In recent years chemotherapeutic treatment of plant viruses have also been reported. It has been demonstrated that *X*-virus of peach could be destroyed by soaking the infected buds in appropriate concentrations of solutions of hydroquinone, urea and sodium thiosulphate. 6-azauracil, 2-thiouracil and 8-azaguanine, all analogues of the RNA base uracil, have been found effective against tobacco mosaic and turnip yellow mosaic.



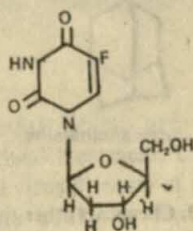
Thymidine



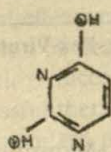
5-Bromodeoxyuridine



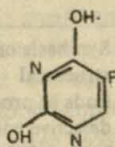
5-iododeoxyuridine



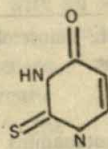
5-fluoro-2-deoxyuridine



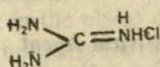
Uracil



5-fluoro uracil



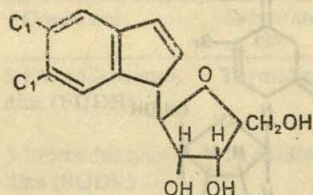
2-thiouracil



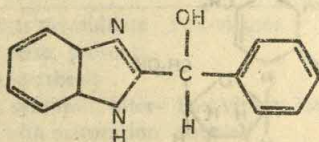
Guanidine Hydrochloride

FIG. 9.2. Base analogues used as chemotherapeutic agents.

In recent years several inhibitors of virus multiplication including antibiotics have been tried, without success, in curbing plant diseases caused by virus. However, one encouraging report by Raychoudhuri and his associates indicated that plant growth regulators like endole acetic acid reduce the multiplication of most viruses as potato viruses X and Y and tobacco mosaic viruses in their tasks.



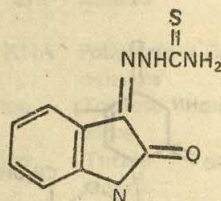
Benzimidazole,



2-alpha-hydroxybenzyl benzimidazole



1-Adamantanamine



Isatin-beta thio semicarbazone

FIG. 9.3. Chemicals other than base analogues use as antiviral agents.

TABLE 9.8. CHARACTERISTICS OF SOME ANTIVIRAL CHEMICALS

| Compound | Substitute For | Influence | Used against |
|--------------------------------|----------------|---|------------------------|
| Isatin beta-Thiosemi-carbazone | Uridine | Synthesis of late viral structural proteins; leads to production of defective particles | Pox-Viruses |
| 1-Adamantanamins | Uridine | Uncertain; probably prevents release of viral nucleic acid | Myxoviruses |
| Polymocinic acid | Uridine | Induces production of interferous | Myxo: Paramyxi Viruses |

Radioactive Phosphorus as Antiviral Agent

It has been possible to utilize radioactive phosphorus (P^{32}) to inactivate nascent viral nucleic acid and consequently the virus particles. Radiophorus of very high specific activity is allowed to be incorporated into nucleic acid molecules during virus replication as a part of the phosphodiester linkages between adjacent nucleotides. Gradually P^{32} decays emitting β particles and get converted into S^{32} . Thus the phosphodiester bonds are gradually converted into sulphodiester bonds. The latter are incapable of holding the nucleic acid strand together. As a result, strand rupture occurs leading to inactivation of the nucleic acid. This inactivation is reflected in the lethality of the virus particles.

Inactivation of viruses like coliphage T_2 , Polyomavirus, poliovirus and coliphage ϕX 174 have been reported by this method.

Interferons as Antiviral Agents

Interferons are natural host specific antiviral agents. Attempts have been made in recent years to use them against specific viral diseases either by directly administering them or by inducing their formation in the host. Artificial induction of interferons production in the host prior to actual infection by virus would enable the host to develop resistance against any future attack. Induction of interferon production by such chemicals like polycytidilic acid and poly iosinic acid have been found useful against several types of neoplastic growth in mice.

Heat Therapy of Plant Viral Diseases

Apart from chemotherapy, viruses infecting plants are known to have been eliminated or inactivated by heat treatment. I.O. Kunkel demonstrated for the first time that several virus diseases of peach, like peach yellow, little peach and peach rosette could be eradicated by treating the planting material at high temperature in the range of 50°C to 55°C. He was also able to eliminate aster yellow virus infecting *Vinca rosea* by treating the plants with temperatures upto 40°C. He also found that effectiveness of the treatment increased with an increase in the duration of treatment. Since then several other reports have come up claiming successful heat therapy of viral diseases. For instance, the successful therapy of ratoon stunting of sugarcane.

It is a standard procedure these days to eradicate the well known disease of sugarcane, namely, ratoon stunting, by treating the 'setts' with high temperature of about 55°C. Similarly, potato leaf-roll virus can be inactivated by treating the tubers with temperature in the vicinity of 40°C for several days. One of the reasons why this disease is not at all prevalent in the plains of Northern India is the fact of storage of potato in the temperature range between 30°C to 35°C for several months (see Kassanis 1967; Smith 1974).

The mechanism of heat therapy is not well understood. It has been suggested that high temperature normally denatures the protein monomers irreversibly, thereby interfering with capsid formation. Probably heat treatment also affects the infective and immunological properties of the virions.

Inhibition of Plant Viruses in situ

Sometimes, when sap transmissible viruses are inoculated onto

healthy susceptible host plants, the virus is prevented from multiplying and producing symptoms in the latter. This is a type of inhibition *in situ* which is probably due to some natural inhibitor. One curious aspect of this phenomenon is that it is the host plant and not the originally infected one that determines the extent of inhibition. For example, dahlia mosaic virus is sap transmissible from infected to healthy *Zinnia elegans* but not from infected *zinnia* to healthy dahlia.

It is now widely recognised that there are specific inhibition, often natural secondary metalolites, present in the plants which prevent sap transmission of infectious viruses. For instance, tannins present in the sap of rosaceous plants are known to inhibit virus infection by precipitating it out on contact. Unidentified inhibitors have been reported from cucumber and rice plant extracts (see Smith 1974).

Chapter Ten

VIRUSES AND HUMAN CIVILIZATION

Existence of life on this planet has been and continues to be the basic imperative behind the phenomenon of viruses. The origin of viruses and in all probability even their specific existence and capacity for their propagation, cannot be thought of without associating living organisms with them. They are partners in a biological model for co-existence, which often is in their favour. Implicit in such co-existence lies the impression that there is operative an interaction between the viruses and their hosts, an interaction that must be considered emerging as a resultant of the 'influence' of the virus and the response offered on the part of the host (These interactions have been discussed earlier in Chapter Eight).

The nature of the effect of this interaction on the living world is difficult to evaluate. Our knowledge mainly relates to the human civilization. The human beings and the plants, animals and microorganisms relevant to the civilization they have created. Scientists have not only evaluated these influences, they have also endeavoured to find out ways and means to apply the information gained therefrom to the benefit of this civilization of ours. Thus, we have come to know about the malevolent influences of these agents, like disease and death, as well as about their application in other fields like industry and medicine. In the following paragraphs, we propose to look into the main features of these applied aspects of virology.

VIRUSES AND DISEASE

Historically, viral diseases and their effects are well documented. All the old civilization, like the Indian, the Chinese or the Egyptian, invariably note the periodic occurrence of such viral diseases as common cold and influenza. Chinese literature describes prevalence of small-pox around 1000 B.C. More and more of such references can be seen in later day documents of many a countries.

Most of the viral diseases known today have, however, been detected comparatively recently, especially in the last two hundred years. Small-pox was considered to be one of the most devastating of disea-

ses, affecting the populace of all the countries of the world. Influenza is known to occur in epidemic form round the world with a regular frequency. Poliomyelitis and encephalitis are other two dreadful diseases, affecting millions of people every year. Some viruses are known to cause cancer.

Plant diseases are also well recorded. Tobacco mosaic is doubly important because of its connection with the discovery of viruses. Tulip 'break' disease, which caused variegation in tulip flower petals, was very well known during the fifteenth, sixteenth and seventeenth centuries. In fact such a change was well cherished by the populace in many European countries.

In recent years, occurrence of new potentially dangerous diseases have been reported. For example the outbreak of serious haemorrhagic fever in Sudan during 1975 and 1976 have been linked to viruses (see Pattyn *et al.* 1977). The challenge and the problem posed by viruses, recognised and potential, are thus quite formidable and demands all embracing attention from the human race.

VIRAL DISEASES OF PLANTS

More than three hundred viral diseases of plants are on record. Several of them are listed in Table 10.1. Viral diseases of plants are known according to the prominent symptoms appearing on the respective hosts. Viruses attack many important crop plants, particularly those belonging to the family *Solanaceae*. Several of these diseases are prevalent in India. However, none of them causes any serious damage to the yield of the crop. Important cereal crops appear to be the least affected.

Tobacco Mosaic

Leaves of plants infected with this disease develop downward curling accompanied with all sorts of distortion. Generally, the younger leaves are affected. Chlorosis also develops; a number of dark green areas appear first, developing later into irregular blisters. Malformation of aerial parts and development of yellowish blotches are two accompanying symptoms.

Tobacco mosaic virus is extremely stable and perennates in plant debris, tobacco refuse and even in cured tobacco. It can retain infectivity even after two decades. Normally, the disease is mechanically transmitted, by mere contact, both direct and indirect. Hands and implements of farm workers are the chief source of infection. However, the virus is also sap transmissible. Sometimes insects such as,

TABLE 10.1. VIRAL DISEASES OF PLANTS

| Disease | Virus | Severity of Incidence |
|---------------------------------------|--------------------------------------|-----------------------|
| Tobacco mosaic | Tobacco mosaic virus | Endemic |
| Tobacco leaf-curl | Tobacco leaf-curl virus | Endemic; sporadic |
| Potato mild mosaic | Potato virus-X | Endemic |
| Potato rugose mosaic | Potato virus-X and Potato virus-Y | Endemic |
| Potato leaf-roll | Potato leaf-roll virus | Endemic |
| Potato crinkle | Potato virus-X and Potato virus-A | Endemic |
| Tomato leaf-curl | Tomato leaf-curl virus | Endemic |
| Tomato bunchy top | Tomato bunchy top virus | Endemic |
| Turnip yellow mosaic | Turnip yellow mosaic virus | Endemic |
| Golden yellow mosaic of bean | Golden yellow mosaic virus | Endemic |
| Cucumber mosaic | Cucumber Mosaic Virus | Endemic |
| Abutilon Mosaic | Abutilon Mosaic Virus | Endemic |
| Little Leaf of Brinjal | Brinjal Little Leaf Virus | Endemic |
| Papaya Mosaic | Papaya Mosaic Virus | Endemic |
| Stunt of Cardamom | Cardamom Stunt Virus | Endemic |
| Ratoon Stunt of Sugarcane | Ratoon Stunt Virus | Endemic |
| Bunchy Top of Banana | Banana Bunchy Top Virus | Endemic |
| Little Leaf of Cotton | Cotton Little-Leaf Virus | Endemic |
| Rosette of Groundnut | Groundnut Mosaic Virus | Endemic |
| Tristeza (Quick Decline) of Citrus | Citrus Tristeza Virus | Endemic |

Myzus persicae are known to act as vectors under laboratory conditions (Pirone 1967)

Tomato Leaf-Curl

It is one of the major diseases of tomato, particularly in India. The main symptom of the disease is drooping and curling of leaves due to inward rolling of leaflets along the mid rib. Leaves also become thickened, crisp and yellowish in colour. Plants become stunted and look rigid. Fruits when formed are small, and ripen prematurely. Affected plants eventually die, the immediate reason being death of the roots. The virus is vector transmitted. White fly (*Bemisia tabacci*) is normally involved. It is also known to infect related Solanaceous plants, like tobacco.

Potato Leaf-Roll

This is one of the important diseases of potato to occur in India. The diseased plants show pronounced inward rolling of the leaves.

Symptoms are most striking during the declining period of plant growth. Usually, a large number of plants get infected and more often than not, the disease assumes epiphytotic nature. Loss in yield is also considerable. The virus is vector-transmitted, *Myzus persicae* playing the role of vector.

Potato leaf-roll is one of the diseases known to be caused by mixed infections. In this case, two plant viruses viz., potato virus-X and potato virus-Y are known to be involved. Although both of these viruses are capable of infecting the plants independently, the particular disease symptoms appear only when both infect simultaneously.

Pumpkin Mosaic

Among the viral diseases of cucurbits, pumpkin mosaic is perhaps the major one to occur in our country. Chief characteristics of this disease are, extreme mottling or irregular variegations of dark green and yellowish green areas and wrinkling of younger leaves. Sometimes, the wrinkling becomes so pronounced that the upper portions of the infected plants look like a small cabbage. The yellowish green areas soon become well spread throughout the leaf surface. Affected plants also show general stunting. Few fruits develop, and those that do, are often with wart like growths. Spread of the disease under field conditions is extremely rapid, particularly in the summer and the rainy seasons. The virus is spread through the agency of insect vectors like *Myzus persicae*.

Tulip 'Break'

Historically, this disease is important because of its being the first to be on record. The disease was so called because of the colour changes that occurred on the petals of the infected plants. The resultant variegation gave the flowers a fantastically wide range of aesthetically beautiful shades. Cultivation of broken tulips became a craze with the people in France and Holland during the sixteenth and the seventeenth centuries; a broken tulip was often worth a fortune. A considerable amount of money used to be spent in growing these flowers, and in acquiring them.

One curious and interesting aspect of this disease was its gradual disappearance without any human effort. Rather, the reverse is true, if we consider the money and energy spent on cultivating them. Although some bulbs carrying the disease are still cultivated, none of them develop the characteristic 'broken' flowers. Apparently, the deleterious effects of the infection slowly but surely suppressed the normal vigour of the bulbs, leading to the gradual deterioration in their

normal behaviour. In fact, even during the height of the craze for such 'broken' tulips, horticulturists growing them were not oblivious of the fact that the plants with such flowers showed somewhat lessened vigour. It is possible that the lowered vigour in its turn might have resulted into the loss of capacity to produce the desired variegations. All the craze for 'broken' tulips is now a thing of a romantic past.

VIRAL DISEASES OF ANIMALS AND HUMAN BEINGS

Diseases of human beings and animals are comparatively better studied. Some of these are listed in Table 10.2. A few of these, like polio and encephalitis, are quite harmful, while a few others are innocuous. We will consider some of them briefly.

TABLE 10.2. VIRAL DISEASES OF ANIMALS AND HUMAN BEINGS

| <i>Disease</i> | <i>Virus</i> | <i>Severity</i> |
|----------------------------|---------------------------|----------------------|
| <i>Mammals</i> | | |
| Small-Pox | Pox-Virus (Variolla) | Epidemic |
| Poliomyelitis | Poliovirus | Epidemic |
| Influenza | Influenza virus A | Epidemic |
| Measles | Measles virus | Epidemic |
| Yellow Fever | Yellow fever virus | Epidemic |
| Encephalitis | Encephalitis viruses | Epidemic |
| Infectious Hepatitis | Hepatitis virus | Endemic |
| Viral Bronchitis | Parainfluenza virus | Endemic |
| Common cold | Rhinoviruses | Endemic |
| Herpetic keratitis | Herpes virus | Endemic |
| Viral enteritis | Echovirus (type 20) | Sporadic |
| Mouse mammary tumor | Mouse mammary tumor virus | Sporadic |
| Myxomatosis (Rabbit) | Papilloma virus | Sporadic; Endemic |
| Hydrophobia (Rabies) | Rabies virus | Sporadic |
| <i>Others</i> | | |
| Viral | | |
| Psittacosis (parrot fever) | Psittacosis virus | Epidemic |
| Chicken sarcoma | Rous sarcoma virus | Sporadic |
| Silkworm jaundice | Nuclear polyhedrose virus | Endemic |
| Granulosis of lepidoptera | Granulosis virus | Endemic |

Influenza

One of those notorious diseases to scourge human civilization, is the influenza. Its viral origin was traced in 1933 by Andrews and his associates. This disease is known to occur in epidemic form involving

several countries at a time. The frequency of occurrence is also very regular. In 1919-20 almost half the world population was affected. In India this disease has been particularly prevalent during the last decade. Though mortality rate is low, large scale epidemics (pandemics) often lead to severe casualties. According to one estimate, the post World War I pandemic led to the death of about twenty million persons.

The prominent symptoms of this disease are, high fever associated with severe body ache, both of which persist for a number of days and then gradually disappear. Another feature to be observed is the extremely weakened condition of the patient. A severe attack could easily become fatal.

Its chief means of dissemination is person to person contact. Coughing and sneezing by the infected person could cause airborne transfer of disease from a diseased to a healthy individual. Sudden appearance of the disease in an unaffected area can often be traced to recent arrival of cargo of persons from infected areas.

One interesting aspect of this disease is the existence of genetic variability in its causal agents. All the great epidemics of influenza have shown distinctive features. It is now certain, on serological evidences, that all of these epidemics were caused by different varieties of influenza virus. One never knows when a new variety of the virus may appear and cause a new epidemic, probably of more serious nature than those occurring earlier. Concerted efforts by various governments and the United Nations organisations, particularly the World Health Organisation, are underway to understand, prevent and control the disease.

Small-Pox

Considered to be the most deadly of viral diseases, small-pox is confined exclusively to man. It is one of the oldest diseases to be chronicled. Chinese records claim its occurrence as early as 1000 B.C. It has an extremely high lethality rate, about 25 per cent. Although successfully eradicated from temperate countries, it is widely prevalent in the tropics. Also, the appearance of the disease is quite regular.

Clinical symptoms of the disease include fever, body ache and, ultimately, appearance of pustules on the skin. These pustules dry up in due course and form crusts, which act as the chief source of infection. The disease is disseminated through direct contact with the source of infection. Apart from the infected person and the dried crusts, items of personal use, like bed-linen, books and utensils can act as sources of infection. One potentially dangerous source of infec-

tion are the persons immunized against the disease but carrying in-apparent infections.

In recent years, the disease is being gradually brought under control. Artificial immunization against the disease has proved extremely effective. Using attenuated strains of carefully selected viral strains as the immunogen, vaccines are produced in different countries. Timely immunization prevents the disease from spreading. Other preventive measures include isolation of the patient to remote localities. In recent years, chemotherapeutic measures are also being undertaken; *N*-methyl isatin thiosemicarbazone, a drug that can prevent viral multiplication, has been used against certain pox-virus caused diseases with partial success. However, it has proved ineffective against small-pox.

Viral Hepatitis

As the name indicates, this disease is a manifestation of viral infection of the human liver. Viral hepatitis is essentially a tropical disease but its occurrence in relatively colder environs is not unknown. The disease occurs in minor or even major epidemics. In India several instances of viral hepatitis epidemics are on record; Delhi itself witnessed a major epidemic in 1954.

There are two types of viral hepatitis, namely, infectious and serum hepatitis. They are now established to be caused by two separate viral strains, IH (type *A*) and SH (type *B*) respectively. There is etiological difference between the two. The former (type *A*) strain has a relatively shorter incubation period (15 to 45 days) as compared to the latter (type *B*) strain which has a longer incubation period (30 to 120 days). The former strain is responsible for epidemic hepatitis while the latter results in sporadic cases of hepatitis.

Symptoms associated with both types of hepatitis are identical in general. The disease occurs in all age groups. The symptoms include lassitude, passing of coloured urine, slight fever, inflammation of liver and ultimately development of yellowing in majority of cases. After a period of two weeks or more, the symptoms generally subside. During the period of disease liver functioning becomes highly abnormal. Normalcy, however, returns as other symptoms subside.

Pathologically, the disease can be acute and some times persistent. It can be non-fatal as well as fulminant and fatal. In both types degenerative changes gradually occur in the liver cells. In many cells the entire cytoplasm gets converted into shrunken bodies referred to as Councilman bodies (Plate XIV). Entire regions of liver undergo massive necrosis, particularly in fatal types.

Infectious hepatitis virus is water borne and is transmitted via con-

taminated drinking water. Suitable water treatment measures, particularly in urban areas, must, therefore be regularly adopted in order to prevent the occurrence of this disease. Mode of transmission of serum hepatitis is not fully established but evidences indicate it to be orally transmitted.

Prophylactic and therapeutic measures against viral hepatitis are still to be perfected. Research is going on for development of vaccine against this disease. In India considerable progress in this regard has been achieved at places like. Haffkine Institute at Bombay, All India Institute of Medical Science at New Delhi and the School for Tropical Medicine in Calcutta.

Poliomyelitis

This disease is also very widely spread, and occurs often as an epidemic. The virus causing the disease affects the alimentary canal and latter, the spinal chord. Infection of the latter leads to eventual paralysis, especially of the limbs. Children generally are more adversely influenced than older people, in whom the effects are mostly mild. For this reason, the disease is also known as 'infantile paralysis.' It has been estimated that a very sizeable portion of the world population has been affected by this virus one time or the other. The famous US President Mr F.D. Roosevelt was seriously handicapped by the effect of this disease.

During the last two decades extensive investigations have been carried out on this disease. Researches were greatly facilitated by the ability of scientists to grow the virus in tissue cultures using tissues other than nerve tissues. It became possible to obtain them in the pure form. Consequently efforts were made towards development of vaccines against this disease. These efforts were crowned with success when Jonas Salk was able to develop an effective vaccine against the disease during the late fifties. Oral vaccines were produced by A. Sabin at about the same time. These vaccines are used extensively in preventing the disease and it is a routine practice these days to inoculate the newly born babies with these.

Rabies

Rabies is one of the earliest viral diseases of man to be recognised. Although history records several instances of rabid (Latin *Ribidus* means mad) dogs terrorizing human settlements, its infectious nature could be recognised only in the early years of the nineteenth century. Much later, during the 1880's Louis Pasteur suggested that the responsible etiological agent was not a bacterium; this was to be

confirmed later, and the viral origin of the disease definitely established, by Negri and others in the early years of the present century.

Rabies virus can infect a wide variety of mammals, both wild and domesticated. Dogs, cats and cattle are particularly susceptible. Wild mammals, like foxes, squirrels and bats serve as a source and reservoir of the dangerous infectious strains of the virus. Man contracts rabies through the bites of already infected mammals, particularly the domesticated ones, like dogs. Man, however, does not normally transmit the disease. Incidence of the disease has been going down gradually in many countries. However, in India its prevalence is relatively greater. This could primarily be due to comparatively less stringent public health measures.

Pathologically, rabies is a form of encephalitis. The spinal chord and the brain are severely affected in a diseased person. Both show neuronal degeneration. Intracellular inclusion bodies or Negri bodies develop in them. Clinically, the disease becomes recognizable with the development of increased muscular tension or spasm concurrently with difficulty in swallowing. The spasmodic contractions are more severe and painful when the muscle comes in contact with liquid. Often, mere sight of a liquid will induce contraction of throat muscles, hence the common name of the disease, *hydrophobia*. A fatal outcome of the disease is considered inevitable if suitable prophylactic measures are not immediately undertaken. But even then approximately 50 per cent of the patients ultimately fail to recover.

Effective preventive measures include isolation of the source of infection and vaccination of potential victims. Restriction of dogs and other domesticated animals and limitation on spread of wildlife have proved useful. Prophylactic immunization of dogs is practised all over the world. Phenol inactivated vaccines and several types of infectious (calternated) vaccines have been developed and used successfully in prophylactic treatment of human beings. The duration of treatment is known to vary with the type of vaccine.

Kyasanur Forest Disease (KFD)

This disease is probably the most important of viral diseases of animals to have been worked out in India. It was reported for the first time from Kyasanur forests of Mysore state (now Karnataka) in 1957. In that year it was noted that monkeys inhabiting that forest were dying in large number due to some unknown reasons.

Investigations by the scientists of the Virus Research Centre, Poona revealed that the sick monkeys were invariably infected with a virus. They were successfully able to isolate the virus and confirm its infec-

tive properties. Further, it was shown by them that the same virus was also present in a species of tick. This tick was identified as *Haemaphysalis spinigera* and was reported to be parasitic on a large number of animals including monkeys and man.

It was also found by these investigators that simultaneously with the appearance of the disease in monkeys, the human population of the area was affected by a typhoid like disease. The same virus as was isolated from the infected monkeys and the parasitic ticks were also found to be present in diseased human beings.

The disease subsequently came to be known as Kyasanur Forest Disease. During the last decade and a half, extensive investigations by Dr T. Ramachandra Rao and his colleagues at the Virus Research Centre have brought out many interesting aspects of this disease. It is now known that the virus is definitely vector transmitted. It is also interesting to know that various hosts that are infected by this virus cover a wide range from birds to human beings. Another important aspect of the infection is that different hosts are infected through the agency of different stages of the same vector, *i.e.*, the tick *Haemaphysalis spinigera*, which is obviously parasitic on all these hosts. The details of the infection cycle from one host to the other is now almost clear.

The disease is endemic (restricted) to a very small region of about 600 sq. miles near and within the Kyasanur Forests. It is rather surprising that the disease does not spread out to the nearby areas within the state of Karnataka itself. However, the virus has been reported to be present in other isolated regions of India, like Kutch in Gujarat. No satisfactory answer to the question of such restricted appearance of the disease is as yet available. It is possible that some specific migrating hosts like birds and bats help in transmitting them year after year. In recent years efforts at controlling the disease have been taken up. Vaccination and vector control have been particularly successful.

Myxomatosis of Rabbit

This is one of the important viral diseases of animals. It was first detected during the last decade of the nineteenth century in Uruguay. Since then it has been recorded from the whole of the American sub-continent. Its viral origin was demonstrated by Dr R.F. Shope in 1933.

The symptoms of the disease may vary in natural conditions, depending upon the host. Initially, infection leads to the production of benign tumours, which in certain cases, slowly but surely progress towards malignancy. For instance, the strain of this virus infecting the common wild rabbit of Brazil causes the development of benign

tumors under the skin only. However, when the same virus was made to infect the European rabbit, there was widespread malignancy all over the body with a virtual cent per cent mortality rate.

The virus is spread mechanically, with or without vector help. Certain species of mosquitoes are known to play an important role in their transmission. Rabbit flea is also known to act as a vector in Europe. Infection by a particular strain often changes the susceptibility of the host. Some host types could be genetically resistant to the virus.

This disease has attracted considerable attention because of its use in the biological control of rabbit pests in Australia. Also, because of its extremely lethal nature, it acts as an effective selective mechanism for genetic resistance in rabbits.

VIRUSES AND INDUSTRY

Applications of virology in industries are considerable. Dependence of an industry upon the viruses could be total; that is to say, the entire set-up could be based on efficient production of virus dependent products. Alternatively, it may be partial, in which case the industry may become indirectly affected, and only in certain aspects of the operations. Production of vaccines and the influence of actinophages on antibiotic industry would exemplify the former and the latter respectively.

Vaccine Production

Virus based vaccine production is one of the major industrial endeavours related to medicine. These days such vaccine production is a routine affair in most countries. The operations include cultivation of specific viral strains, pathogenic for a particular disease, under controlled conditions. Suitable cultural media are developed for the purpose. In the earlier days, laboratory scale operations were more prevalent. However, gradually, continuous culture techniques utilizing more sophisticated fermentation technology, have replaced the earlier modes.

After cultivation, virus particles are separated from the residual debris in the media, by utilizing techniques like graded ultra-filtration. Stock suspensions are then prepared, and the particles enumerated. The suspensions are then finally treated for the production of the desired type of immunogen; they are either killed or inactivated, by applying heat or by treating with suitable chemical agents, such as, phenol or formalin. Under certain conditions 'attenuated' viral strains

are used as vaccines. There are various techniques to produce such strains, chief amongst which are (1) high temperature cultivation, (2) ageing of the culture, (3) drying, and (4) careful selection of mild strains.

Thus processed, virus suspensions are ready to be used as vaccines against specific diseases. Ability of virologists to cultivate viruses in tissue culture, that too involving tissues other than those normally affected has been the very basis of large scale development of viral vaccines. Various aspects of vaccine production involve considerable amount of money and human endeavour. In India, Haffkine Institute, Bombay and the Bengal Immunity Centre in Calcutta are two of the principal institutions engaged in vaccine production in commercial scale.

Other Involvements

Organised industry has to make considerable effort every year to prevent viruses from adversely affecting some important industrial micro-organisms. *Streptomyces gresius*, the actinomycete producing the antibiotic streptomycin, is known to be adversely affected by actinophages, a type of bacteriophage. Therefore, unless utmost precautions are adopted while cultivating the micro-organism, the phage might prove quite a costly nuisance. Precautionary measures are also undertaken during the cultivation of other antibiotic producing species of *Streptomyces* used in industry.

Other industrially important micro-organisms to be adversely influenced by viruses include *Streptococcus lactis*, which is used for 'souring' of milk in dairy industry. Unless carefully excluded from the cultivation vats, this process is severely hampered with, causing considerable losses to the industry. Similarly, some viruses known as zymophages, are virulent towards several species of *Saccharomyces* which are used in bakeries and breweries all over the world. Planned efforts are made to prevent their infection. In both the above cases, development and use of genetically resistant strains of the micro-organisms have been found to be quite effective in combating the menace.

Viruses also concern the agriculturist. Their major influence, apart from their causing some diseases of crop plants, involves the effect of bacteriophages affecting nitrogen fixing bacteria, like *Azotobacter* and *Clostridium*. Some viruses, the cyanophages, affect blue-green algae too. As is well known, these algae are also important group of nitrogen fixers, particularly in tropical rice fields. It can, therefore, be safely presumed that any adverse influence of the cyanophages on the blue-

green algae would affect the nitrogen economy of soils harbouring them.

Bacteriophages and cyanophages are also known to influence the process of sewage disposal. This process is primarily based upon the activity of certain bacteria, such as *Pseudomonas*, *Micrococcus*, *Cytophaga*, *Enterobacter*, *Clostridium* etc., and a few other organisms including the blue-green alga *Plectonema*. Any harmful influence on these organisms, as may be due to phage infection, is bound to affect the entire process.

VIRUSES AND BIOLOGICAL CONTROL

Biological control of any natural phenomenon implies that some specific biological behaviour of one organism is manipulated in such a manner as to be useful in the prevention of harmful influences of other organisms. Viruses, because of their obligate parasitic nature, infect a wide variety of organisms and parasitize on them. Often, they cause serious diseases of the hosts, invalidating or completely liquidating them. Some of these host organisms are often themselves harmful *vis-à-vis* human endeavour. From that point of view their complete eradication or at least abatement of their activities, should be considered desirable. It is here that human ingenuity has made viruses play an important role.

Exploitation of viral diseases in eradicating harmful organisms has been most prevalent in the case of insect pests. As long ago as in 1915, a virus disease was suspected to be the agency responsible for spontaneous eradication of the caterpillars of Gypsy moth, an insect pest occurring in forests of Southern Europe. However, the first clear evidence of the control of a pest insect was reported during the 1930s. At that time European spruce-sawfly became a serious pest in Canada and the USA, destroying thousands of acres of spruce (*Picea* spp.) trees. A chance introduction of a nuclear polyhedrosis virus from Europe checked the sawfly population effectively and it gradually ceased to become a serious pest. Since then considerable attention has been paid to the viral control of insect pests, and, in recent years, it has become one of the most rapidly expanding fields concerning pest control.

Insects are mainly infected by two groups of viruses, namely, the polyhedroses and the granuloses. In the former, hundreds of particles are contained in each polyhedral crystal, where as in the latter each crystal contains only one virus particle. Both types spread rapidly in the insect populations susceptible to them, and, therefore, are poten-

tially valuable as insecticides. Particular attention has been paid to the nuclear polyhedrosis viruses, which not only spread rapidly but also are more devastating.

Eradication of insect pests by artificially inoculating them with specific infectious viral strains have been widely attempted in recent years. Usually, viral suspensions, either prepared from infected insects, or of particles cultivated in the laboratory on specific host larvae, are sprayed on crops infested with different larval stages of the pest insect. To obtain satisfactory results, prior knowledge of the etiology of the viral disease associated with a particular pest, is considered essential. Such information also facilitates cultivation of the virus infecting the pest insect in artificial cultures.

The nuclear polyhedrosis viruses which cause most of the diseases associated with the larval stages of the members of Lepidoptera (butterflies and moths), have been utilized in many a cases. Control of the pest insect *Kotochalia junodi*, which causes extensive defoliation of *Acacia mollissima* (important for its tannin rick bark) in Africa, is a case in point. Several other polyhedrosis viruses infecting different pests have been cultured in the laboratory and have been found to be quite effective in field trials. Two such examples are the nuclear polyhedrosis viruses of Cotton bollworm (*Heliothis zea*) and Cabbage worm (*Trichoplusia ni*). Similarly, a granulosis virus has also been effectively used against insect pests affecting pineapple plantations in Great Britain. Similar attempts involving other insect-virus systems are in progress in different parts of the world.

Viruses have also been useful in eradicating pests other than insects. The most noteworthy example is the control of rabbit pest in Australia by infecting the animal with myxomatosis virus. There was almost total eradication of the pests. One interesting aspect of this virus is its ineffectiveness against rabbit pests in South America.

These approaches towards eradicating tiresome and harmful pest are still in their formative stages. The principles and techniques involved with different pests are different. This necessitates intensive research in each case. Further, the different stages in each operation viz., preparation of viral sample, spraying under field conditions, infections of host, persistence of infection etc., are still to be perfected in the majority of cases. Also induction of diseased conditions in the insects by other means should be made possible. As has been suggested by Dr K.M. Smith, we should investigate for latent viral infections in the various pests and should explore ways and means to induce them into virulence. Possibilities of infecting the pest with virus from any readily available source should also be considered.

Perhaps, all this would mean, as pointed out by Dr J.R. Norris, a forced change in the ecology of the insect. Be that as it may, success in such efforts is bound to usher in a significant step towards virus mediated biological control.

Phage Typing of Bacteria

It has been conveyed earlier that viruses exhibit considerable host specificity. Bacteriophages are no exceptions. Host specificity is sometimes wide in the sense that the same virus shows the capability of infecting two different host species which may belong, however, to the same group or class. For instance, rabies virus infecting man as well as dog. On the other hand there are many instances of extremely restrictive host specificity. Several strains (varieties or races) of bacteriophages are known which would infect only one particular bacterial species or even only one of its strains. Such specificity could be existing naturally but more often than not specific strains (both of the virus and the host bacterium) are artificially induced and maintained in the laboratory.

This phenomenon of extremely restrictive host specificity can be used with advantage in recognising and identifying bacterial types, particularly those which might be pathogenic. It often becomes a problem to distinguish between pathogenic and non pathogenic forms as these behave in exactly identical manner excepting their response towards specific phages. Therefore, this procedure of identifying bacteria by observing whether they are specifically infected and consequently lysed by a specific bacteriophage is known as *Phage typing*.

Let us take the example of phage typing of the typhoid bacteria, *Salmonella typhi*. In the first place, bacteriophages specific for a few selected strains of *Salmonella typhi* are propagated in the laboratory. By successive propagation for a few generations, these phages become specific to a degree for a particular strain of the bacterium. However, this specificity becomes absolute for that strain when the phage suspension is sufficiently diluted. It has been observed that if this dilution is not achieved or maintained, there are chances of other strains of *Salmonella typhi* getting infected and lysed as well. This level of dilution is known as the *Critical dilution*.

After this critical dilution is attained, the unknown and suspected *Salmonella typhi* cultures are mixed with a different known type of *Salmonella typhi* bacteriophage on petri plate having suitable agar based medium. If the unknown bacterial strain gets infected and lysed only by the bacteriophage specific for the pathogenic strain of *Salmonella typhi* (which was determined previously), then it becomes clear

that the unknown strain is indeed pathogenic.

Bacteriophage types of several strains belonging to different pathogenic bacteria have been developed. One of the first highly specific phage to be developed was a typhoid bacteriophage designated as *Typhoid phage A*. Several other typhoid phages are designated as *B, C, D, E, F* and so on. Similar typing systems have been developed for other species like *Staphylococcus* and *Shigella*.

Phage typing is widely used for medical diagnosis and in industry. In Delhi, well developed phage typing facilities are available in Lok Nayak Jay Prakash Narayan Hospital, New Delhi.

Identification of Latent Infections

One important aspect of applied virology has been recognising and identifying unknown and latent infections. Viral serology, particularly such tests as Precipitin test and complement fixation test are almost routinely used in detection of suspected viral infection. Successful detection in advance can be of immense prophylactic value.

Latent infection in plants are recognised by inducing the suspected virus (by sap transmission) sources to produce characteristic symptoms on certain specific hosts called the Indicator plants. These plants are so named because of their prompt ability to produce characteristic symptom of a disease on sap transmission. If the symptoms produced are identical in case of inoculation by known and suspected viral forms, their identity and similarity becomes self-evident. *Nicotiana glutinosa*, *Phaseolus vulgaris* var Pinto and *Chenopodium amaranticolor* are widely used indicator plants for number of plant viruses.

Miscellaneous

Apart from the involvements outlined above, viruses are of considerable importance as research material, used in various laboratories all over the world. Their role in fundamental researches attempting to elaborate the basic nature of the life process, can never be over emphasised. Some of the stoutest pillars of molecular biology and medicine are based on the knowledge obtained from the study of viruses. Their very biological nature, which remains uncertain as yet, is of unique significance for in it may lie answer to the riddle that confronts us most, the riddle of life.

APPENDIX

GLIMPSSES INTO SOME NEW DEVELOPMENTS IN VIROLOGY

CHAPTER ONE

I. **Viruses as agents causing cancer in man.** The suspicion that viruses may be directly responsible for causing certain types of cancer in human beings now appears to be well founded. Several recent reports conclusively associate Herpes Simplex Virus type 2 (HSV 2) with the occurrence of human cervical cancer (*see Dressman et al.* 1980 and associated references).

II. **New strains of disease causing viruses.** Evidences are accumulating which indicate that newer strains of pathogenic influenza viruses occur in nature spontaneously at a fixed frequency over a period of time. This explains the appearance certain types of influenza in epidemic form after a lapse of even decades. (*See report on An International Workshop on 'Structure and variation in influenza virus' held at Australian National University, Canberra, Australia. Nature* 283, 524-25, 1980.)

CHAPTER TWO

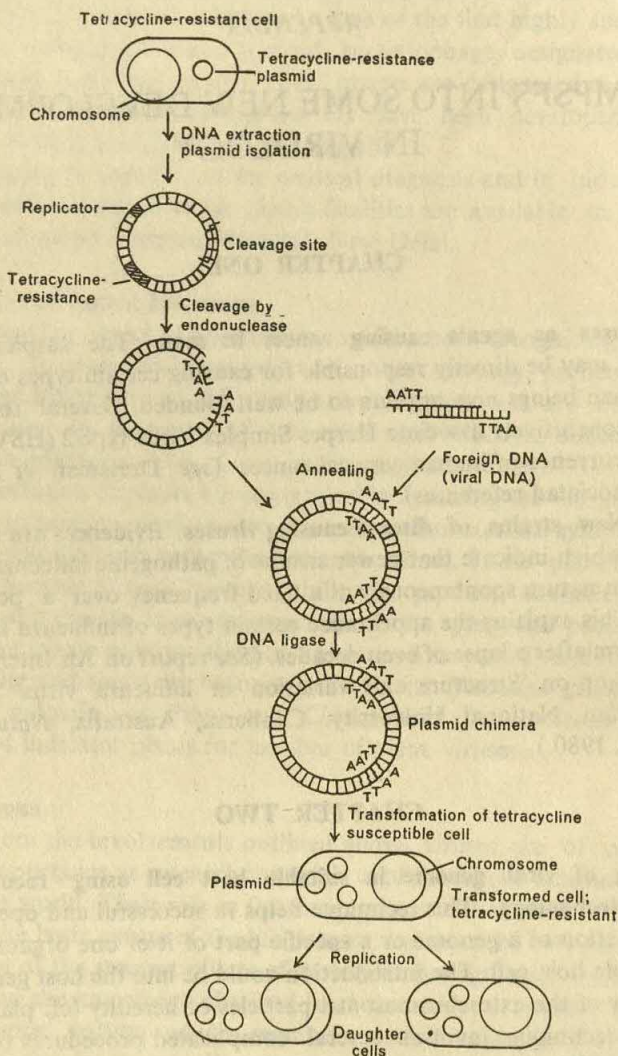
Cloning of viral genome in suitable host cell using recombinant DNA technology. This technique helps in successful and operational introduction of a genome or a specific part of it of one organism into a suitable host cell. The introduction could be into the host genome or into any of the extrachromosomal particles of heredity (cf. plasmids).

The technique involves several complicated procedures (Fig A.I. on page 252). These are, briefly,

(i) exact identification of the genome or part thereof, to be transferred. Knowledge of its nucleotide sequence and its nucleotide mapping is ideal.

(ii) Isolation of the genome by suitable means and if the genome is single stranded, making it completely double stranded and circular.

This is subsequently cleaved into a linear structure with specific restriction endonuclease (e.g., EcoRI.)



(iii) Isolation of the host cell plasmid usually with a specific marker and conversion of these double stranded circular DNA particles into linear stranded ones by specific restriction endonuclease enzyme (e.g. EcoRI).

(iv) Ligation of the linear genome to be cloned with the linear plasmid genome using Ligase enzyme system. The plasmid genome is made circular again, by artificially manipulating that free ends of both the plasmid genome out of the foreign DNA are complimentary, thereby capable if being joined.

(v) Transformation of host cells harbouring the plasmid by manipulating the induction of the ligated genome into it.

(vi) Identification and isolation of host cells carrying the plasmids containing ligated genome to be cloned with the help of marker trait.

(vii) Cultivation of the host cells carrying the plasmids with cloned genome.

Utilising this technique successful cloning of hepatitis B virus (HBV) genome in *E. coli* K₁₂ strain cells has been achieved.

CHAPTER THREE

Nucleotide sequences of more viral genomes. Nucleotide sequences of more viral genomes are being reported. In a recent study (Gailbert *et al.* 1979) determined the complete nucleotide sequence of hepatitis B virus (HBV) genome. This sequence is 3182 nucleotide long. The coding capacities of the two chains are not identical indicating a difference in the amount of nonsense codons in each. Eight open regions able to code for polypeptide chains larger than 100 amino acid are recognised. Region 6 is the largest and comprises about 80 per cent of the genome.

CHAPTER FOUR

Architecture of Tomato Bushy Stunt Virus (TBSV). In a recent report, the architecture of TBSV has been resolved at 5.5Å resolution. The TBSV is a small spherical RNA virus whose particle consists of a single RNA molecule (MW 1.5×10^6), covered over by a protein coat built by 180 subunits (MW 4.1×10^4). The small sphere is a definite icosahedron with the coat protein forming a T=3 icosahedral surface lattice. This design implies that a single polypeptide chain packs in relation to its neighbours in three slightly different ways. The subunit packing of the protein shell is quasi-equivalent (not fully symmetrical). This necessitates a special type of packing of the identical subunits and is achieved by protein subunits having rigid domains (regions) connected by a flexible hinge. Each subunit has a binding site for RNA on its inner surface (see Winkler *et al.* 1977).

Architecture of satellite tobacco necrosis virus (STNV). Recent

reports have thrown more light on the architecture of another plant virus, the satellite tobacco necrosis virus (STNV). One of the smallest known plant viruses, STNV has a single stranded RNA ($MW 1.5 \times 10^6$) covered over by a protein coat composed of 60 identical protein molecules ($MW 21,600$). The complete nucleotide sequence of RNA and that of amino acid of proteins are known. The coat is a regular icosahedron ($T=1$). Details of the arrangement of the polypeptide chains of protein subunits have been worked out. It has been revealed that the polypeptide chains have a three twin helix near the N-terminal end (see Unge *et al.* 1980).

CHAPTER FIVE

I. How do bacteriophage blocks host gene expression while replicating itself? It had been known for a long time that the bacteriophage T_4 shuts off *E. coli* gene products. Further that, in the process the bacteriophage depends entirely on *E. coli* transcriptase and *E. coli* protein synthesis machinery. For some time it was thought that T_4 degrades *E. coli* DNA while protecting its own genome from self destruction by replacing its cytosine with hydroxymethyl cytosine, not recognised by the phage nucleases. However, it is now known that host gene products stop appearing well before *E. coli* DNA is broken down. The problem has, therefore, remained unsolved for a considerable period of time.

Recently, more light has been thrown on the problem. It has been shown that specific T_4 protein bound to *E. coli* RNA polymerase, after T_4 infection, may be a fact in the shutdown of *E. coli* RNA synthesis after infection. It had been shown earlier by Stevens (1972) that four T_4 proteins are covalently linked to *E. coli* RNA polymerase. Two of these are positive control elements regulating the synthesis of the major class of T_4 protein appearing later after infection. Another, about 10^4 daltons in size, apparently inactivates the sigma subunit of RNA polymerase. The fourth protein is most probably synthesised by *alc* gene of T_4 and is responsible for blocking of transcription of cytosine containing DNA. The mechanism of *alc* protein mediated block of transcription of cytosine containing DNA is not convincingly understood. One possibility is that it reduces the affinity of *E. coli* RNA polymerase for *E. coli* and for RNA promoters, causing almost complete shut off of *r*-RNA synthesis. It is also possible that some of the component factors of RNA-polymerase factors are structurally and functionally altered due to phage infection causing the shut off (See Goff. 1977).

Role of ion uptake in suppression of host cell activity. Some recent reports indicate that viral infection possibly leads to change in the ionic environment of the host cell. It has been established that in polio-virus infected cells, absorption of monovalent ions like Na^+ increase considerably. And also that this enhanced absorption is a pre-requisite for shutting off of host cell activities. (Carrasco, L & A.E. Smith 1976; Nature 264, 807) Ca^{++} ions are also known to play a similar role.

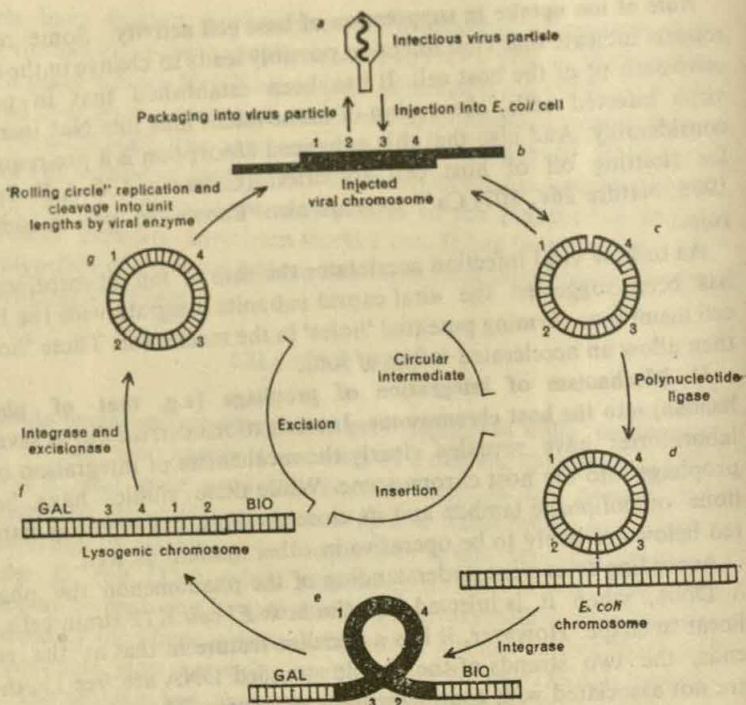
As to how viral infection accelerates the rate of ion absorption, it has been suggested the viral capsid subunits integrate with the host cell membrane forming potential 'holes' in the membrane. These 'holes' then allow an accelerated influx of ions.

II. Mechanism of integration of prophage (e.g. that of phage lambda) into the host chromosome. Investigations carried out at several laboratories have revealed clearly the mechanism of integration of a prophage into the host chromosome. While these studies have been done on coliphage lambda and its close mutants, the events as narrated below are likely to be operative in other systems as well.

According to current understanding of the phenomenon the phage λ DNA, when it is injected into the host *E. coli* K12 strain cells, is linear in shape. However, it has a peculiar feature in that at the two ends, the two strands of the double stranded DNA are free i.e. they are not associated with complementary sequences. These free ends are relatively small (about 12 nucleotide ions) and are referred to as the 'sticky ends'. Another interesting feature of these linear strands is that the two sticky ends are in themselves, apparently, complementary. This feature is established by the fact that, as determined by genetical experiments, the phage DNA soon becomes circular, simply by looping over in such a way that the two free ends face each other. Biochemical evidences indicate that the small gaps between the impaired complementary strands are bridged by the activity of the bacterial enzyme polynucleotide ligase.

The circular phage DNA then gets inserted into the host chromosome at specific insertion sites, which for phage lambda is the position between *E. coli* genes *gal* and *bio*. The insertion is possible because the viral and the bacterial genome break and reunite at specific sites, two ends of the broken viral genome joining the broken bacterial segments. The process is catalysed by the viral enzyme integrase. The *E. coli* chromosome, and hence the cell itself, is now lysogenic for phage lambda. The latter is now in its temperature state and exists as a prophage only.

After many cell generations the integrated prophage could be induced



Insertion and excision of the phage-lambda genes into the *E. coli* chromosome require the action of both bacterial and viral enzymes. As the DNA of phage lambda is packaged in the viral particle (*a*) it is linear and double-helical, except for complementary unpaired segments 12 nucleotides long at the ends of the two nucleotide chains (*b*). In solution this linear form comes to equilibrium with a circular form that has staggered "nicks" 12 nucleotides apart in the two complementary chains (*c*). When viral DNA is injected into the bacterial cell in the course of infection, the two nicks in the open circle are sealed by the bacterial enzyme polynucleotide ligase, so that both chains of the circle are now closed throughout their length (*d*). This circular intermediate then interacts with a particular segment of the *E. coli* chromosome (between the *gal* and *bio* genes). Viral and bacterial chromosomes break and rejoin at unique sites on each partner, so that viral DNA is spliced into the host DNA, a reaction catalyzed by the viral enzyme integrase (*e*). (Note that the gene order in the pro-virus is 3, 4, 1, 2, a cyclic permutation of the viral gene order 1, 2, 3, 4.) The *E. coli* chromosome is now lysogenic for phage lambda (*f*). After several cell generations radiation or chemically active compounds may induce the pro-virus to enter the lytic state. When this happens, the lambda repressor, which has so far blocked the expression of most of the viral genes, is inactivated, allowing the synthesis of the viral enzyme excisionase. Together with integrase, excisionase catalyzes the excision of the provirus from the host chromosome,

(Contd.)

to become lytic. When this happens the inserted prophage is excised specifically from the host chromosome. This operation is catalysed by another viral enzyme called excisionase. Integrase then rolls the linear prophage into a circular one. The latter then acts as a template and produces more viral DNAs. These viral DNA copies are then cleaned by specific viral enzymes to give linear DNA with sticky end. The virus parlides are subsequently produced (*see* Campbell 1976).

III. Assembly of Tobacco Mosaic Virus. Two recent papers have further elucidated the structure and mode of assembly of tobacco mosaic virus. Cambell *et al.*, (1976) reporting the architecture of TMV protein discs at 5Å resolution have shown that the discs consist of two rings each of 17 protein subunits. The rings are stacked on top of each other, partially facing each other, as it were. These make a firm contact at high radius, they are separated vertically at the RNA-binding radius. These features suggested that the RNA might well be intercalated between the two successive rings of the disc.

In another paper Butler *et al.*, (1977) have put forth a configuration of MV-RNA during assembly. It had been reported earlier that assembly of TMV particles from isolated coat proteins and RNA starts not at one end but rather somewhere in the middle, the location of the nucleation region being about 1000 nucleotide residues from the nearer end of the RNA molecule (3' hydroxyl terminus). Assuming the protein assembly to be proceeding in two directions i.e. towards 3' and 5' termini, presence of small free RNA tails should be expected at both the ends of the finished rod. However, this is not so and the free tail is visible only at the 3' end. It is proposed in this paper that complete coverage of uncoated RNA is possible because of a special configuration of TMV-RNA during assembly. Accordingly, when segregated components of TMV reassemble, the uncoated RNA is folded back along the growing rod, probably down the Central hole. Such doubling back of RNA is considered essential for elongation. It is suggested that such a geometry would allow the relevant regions of RNA to be in contact with the binding sites on the protein discs, keeping rest of the RNA free, thus facilitating rapid assembly.

converting it back into the circular form with the original gene order (*g*). The circle of viral DNA replicates, producing multiple copies that are then cleaved by a specific viral enzyme to give rise to the linear form with "sticky" ends (*b*). Each linear DNA segment is then packaged in a virus coat (*a*). When the host cell ruptures, the liberated phages infect healthy cells and the lysogenic cycle begins anew.

CHAPTER SIX

I. Mechanism of transduction. It is well established that induction of a prophage to lytic state involves exact segregation of the prophage of DNA sequences from its insertion location in the bacterial chromosome. And further, that a transducing prophage element carries with it a small length of bacterial nucleotide sequence (exogenote). The mechanism as to how this might happen, particularly in view of the necessity to keep the overall size of the phage genome constant, remained unclear. One possible explanation had been genetic exchange between the two genomes (discussed in the main chapter).

More recent investigations suggest an alternate possibility. Accordingly, the prophage is separated from the host genome through the activity of a viral enzyme excisionase. While normally this enzyme separates out only the prophage sequences, it could, rarely and wrongly cut out a few of the bacterial nucleotides as well. If these nucleotide sequences happen to carry one or more functional gene, the chances of the prophage for acting as a transducing agent becomes on subsequent infecting and lysogenisation in a suitable host quite clear (see Campbell 1976).

II. Self-control of bacteriophage lambda. The genetic mechanism of suppression of lambda genome while it remains integrated with its host chromosome is not fully understood. It had been recognised earlier that repressor protein molecules are synthesised by a few viral genes which block the activity of the rest of the lambda genes. A few recent reports have helped decipher the mechanism to a considerable degree.

It is now established that the repressor is transcribed and then translated from a structural gene *CI*. It has a promoter situated at a distance. A message from the promoter (*Pre*) is essential for *CI* to be active. Again in its turn *Pre* can become active only when two more phage products *CII* and *CIII* are available. These two phage products are not synthesised while it remains in temperate state. Therefore, *Pre* cannot play any role in synthesising the repressor molecules to the desired level, if at all.

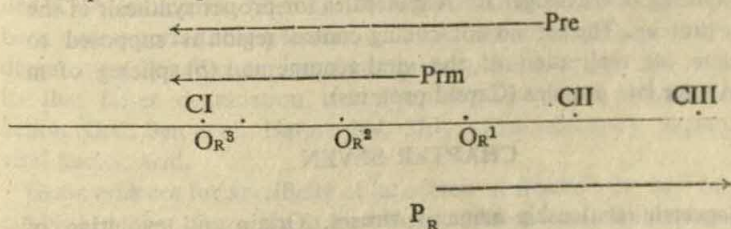
How is then the repressor level maintained? It is now established that a different promoter *Prm* located close to *CI* is needed for this. *Prm* is not a constitutive promoter and needs to be induced. It has now been revealed that small amounts of the repressor itself act as the inducer triggering off *Prm* transcription. It has also been shown that initially, just after infection, large quantities of the repressor proteins are synthesised. However, as soon as the phage genome gets

integrated, the repressor synthesis stops, presumably because of non-synthesis of CII and CIII factors. The residual repressor protein molecules then induce the promoter P_{rm} .

How then does the repressor actually control the phage activities? Investigations have revealed that the repressor protein not only induces P_{rm} but also stops the transcription of another promoter P_R which is active in lytic phage development but remains repressed in a lysogen.

The mechanism by which the repressor prevents other genes of the phage (Lysogen) from being active is also established. It appears that the repressor binds with closely situated operator genes O_R^1 , O_R^2 and O_R^3 . The promoter P_R , responsible for lytic phage development is situated between O_R^1 and O_R^2 . Repressor by binding to these operators prevents RNA polymerase from binding to P_R . The operator O_R^3 has been recognised to be for P_{rm} , the promoter for repressor synthesis. It is rather interesting to note that proper binding of the repressor to operator O_R^1 and O_R^2 leaves a sequence, contained in O_R^3 , which is similar to P_R but opposite in orientation. This is P_{rm} . It is possible that while P_R is blocked P_{rm} is free to interact with RNA polymerase or even with the corresponding DNA sequence, thus initiating CI transcription. It is to be noted that possibly, the promoter P_{rm} signals transcription towards the left because of repressor O_R^1 interaction.

A simplified map of the control region is given below:



III. Nature of the genome of more viruses. Nucleotide sequences and the genetic maps of more viral genomes viz., hepatitis B virus (Gailbert *et al.*, 1979) Simian virus 40 (SV40) (Reddy *et al.*, 1979) polyoma virus (Soeda *et al.*, 1980), have been elucidated in recent years.

Hepatis B virus (HBV) DNA is 3182 nucleotides long, containing eight open coding regions. Gene 5 which codes for the viral surface antigen (HBsAg) is contained in the region 7. Region 6 is the largest of all and comprises about 80 per cent of the entire genome.

Polyoma virus and Simian virus 40, considered identical earlier, are

in fact closely related viruses. Both are double stranded DNA viruses containing 5,292 and 5,226 base pairs respectively.

Polyoma virus codes for 6 known proteins (3 early proteins designated as small, middle and large T antigens and 3 late capsid proteins). The total coding capacity of the polyoma virus DNA in a single frame does not allow coding of all these proteins. Therefore, there exist different coding frames (three in number). In other words, these proteins are coded by overlapping (contained) genes. Investigations have identified the regions of the genome coding for the various proteins.

The Simian virus 40 (SV40) genome is found to be similar to Polyoma genome except that SV40 does not code for middle T antigen and also has duplicated sequence outside of the coding region.

All these studies convincingly establish, what had been done earlier in the case of single stranded DNA virus $\phi \times 174$, that there exist overlapping or contained functional nucleotide sequences or genes thereby demolishing one of the hitherto basic tenets of molecular biology.

Two non-coding regions in the polyoma virus are recognised. These are presumed to be regions which control the genome activity. One interesting feature of one of the control regions is that it lies within the gene responsible for synthesis of early proteins, the T antigens. It is further presumed that this non-coding region might be regulating splicing of messenger RNA molecules for proper synthesis of the early proteins. The second non-coding control region is supposed to regulate (a) replication of the viral genome and (b) splicing of m RNA's for late proteins (Capsid proteins).

CHAPTER SEVEN

Phylogenetic relationship amongst viruses. Origin and evolution of viruses have never been fully understood. A recent paper on the phylogenetic relationship amongst three virus throws considerable light on the problem (Soeda *et al.*, 1980).

On the basis of comparison of the nucleotide sequences of the genomes of three papova viruses, namely Polyoma, Simian virus 40 and the human papov virus, BKV, they have come to the conclusion that these viral species have evolved from a common ancestor and diverged with its host organism. These three virus can easily be isolated from their natural hosts, namely, mouse African green monkey and human tissues respectively.

Analysis of the homologies present amongst the three genomes in

terms of nucleotide sequences of their genomes had revealed considerable similarity amongst the three. Now, by calculating the rate of nucleotide substitution/change in the three genomes the degree of divergence amongst the three was determined. Taking a fixed rate of nucleotide substitution into consideration the probable time at which these genomes diverged from each other could be calculated. The data obtained compared favourably with those obtained from globin protein analysis and also with paleontological and taxonomic evidences. The overall conclusion was that these three viruses had undergone host dependent evolution. Thus when the order Rodentia diverged from the order primate some 80 million years ago, Polyoma virus diverged from SV40 and BKU. Later, approximately 35 million years ago when the sub-order catarrhini diverged from the ancestral primates, SV40 and BKV also diverged from each other.

CHAPTER EIGHT

Mechanism of interferon action. Interferon inhibits the replication of a large number of animal viruses. One probable way how this might be accomplished would be interferon induced degradation of viral messenger RNAs. That this is in fact so, has been demonstrated. It has been shown, for example, that reovirus messenger RNAs are degraded much faster in interferon treated Ehrlich ascites tumour cells than in control cells. Further that presence of additional double stranded RNA molecules (Reovirus contains doublestrand RNA). It has been now reported that presence of ATP further accelerates mRNA degradation by activating interferon protein. Further, it is now reported that faster degradation is a direct result increased endonuclease action (G.C. Sen *et al.*, Nature 264, 370) which selectively degrades viral nucleic acid.

Genic evidence for specificity of interferon production. It has been established that there are at least three different kinds of interferons which can be distinguished on the basis of their cellulose origin and activity. These are: (i) immune or γ interferon, (ii) fibroblast or θ interferon and (iii) leucocyte or σ interferon. Further, there are indications that one particular type of interferon could be the product of two or even three genes and that there could be these genes could be producing three different kinds interferons all perfectly functional [see Robertson M. Nature 285, 358 (1980)].

CHAPTER NINE

New antiviral agents. In recent years several new antiviral agents have come to light. B.N. Gupta and his associates have isolated an antiviral agent, 6MFA, from a fungus *Aspergillus ochraceous*. They have shown that 6 MFA induces very high interferon activity in sera obtained from mice. This high interferon titre sera could than be transferred to healthy individuals making them resistant to attack by Semliki forest virus (Maheshwari *et al.*, 1977 *Acta virol* 21 63).

More recently Pompei *et al.*, (1979) have reported that ammonium salt of glycyrrhizic acid, obtained from roots of *Glycyrrhiza glabra*, as also the extracts of the plant root are strong antiviral agents. They have shown that this drug inhibits the growth of several unelated RNA and DNA viruses such as polio virus, vescicular stomatitis virus and New Castle disease virus [Pompei *et al.*, (1979) *Nature* 281, 689].

CHAPTER TEN

I. Virological evidence for the success of small pox eradication programme. World Health Organization (WHO) surveys confirm that small pox which prevailed the world over during so many previous centuries has been contained. Extensive eradication campaign organised by national and international agencies have apparently stopped transmission of the disease in human population. The risk of natural re-introduction is negligible.

II. Another advancement made in the study of viruses concerns cloning of viral genomes in appropriate host cells. Study of some viruses, like Hepatitis B Virus (HBV), pose problems because they cannot be propagated in cell cultures, the only available source being human serum. By cloning HBV genome in *E. coli* selected strains, an infinite supply of pure HBV genome can be ensured. The cloned and purified genome could be used for various kinds of investigation including finding out possibilities of Vaccine production [Sninsky *et al.*, (1979) *Nature* 277, 346].

III. A similar report relates to successful cloning of cell specific interferon genes from mammalian sources into selected strain of *E. coli* K12 by Japanese workers. It is a remarkable development with far reaching implications with regard to chemotherapy of viral diseases. This achievement would enable large scale economic production of host specific interferon molecules. Also, it would enable one to obtain them in a more purified state than would otherwise be possible.

GLOSSARY

In the following pages a glossary of some relevant terms have been given. In general, a glossary serves two principal purposes. Firstly, it endeavours to provide a definitive explanation of the terms concerned from a technical point of view. Secondly, it also gives an idea about the linguistic derivation of the words in a particular language. An explanatory sentence or two definitely helps the student, particularly when she or he happens to be a beginner in the subject. A knowledge about the derivation of the word, which might have its root in a very different language altogether, should accelerate the process of understanding to a considerable degree. To our mind, the knowledge about the derivation of a word should be particularly helpful to our students, for most of whom English itself is an alien language.

We would also like here to explain certain lexicographic abbreviations used in the glossary. A knowledge of these will be immensely helpful in understanding the relevant terms especially when these have been used in relation to some other terms. The abbreviations used are:

- cf. derived from Latin *confer* meaning compare.
- op. cit. derived from Latin *oper citato* meaning 'cited in the text or work'.
- q.v. derived from *quòd vide*, a Latin phrase meaning 'which see'.
- pre short for prefix, a word or syllable used before another word.
- suf short for suffix, a word or syllable used after another word.

Prefixes and suffixes usually give added emphasis to the main word used after or before them.

Å: Abbreviation for an Angstrom, 10^{-4} of a micron; there are 10,000,000 Angstroms in a millimeter.

Agar (Malay, *agar-agar*): A gelatinous substance obtained from certain species of red algae. e.g., *Gelidium*.

Angstrom: After A.J. Angstrom, a Swiss physicist; equals 10^{-7} millimeter.

Antibody (Greek *anti*, against plus *body*): Gamma globulin type of protein produced in an animal (vertebrates and insects) and by the animal, in response to contact with a substance which is normally foreign to it.

Antigen: Any substance inducing or stimulating the formation of antibody in the cell. Generally proteinaceous or carbohydrate.

Assembly (Latin *assimulare*, to bring together): The phase during replication of viruses in which the process of integration of viral components into complete virus particles takes place.

Autotrophic (Greek *auto*, self plus *trophe*, to nourish with food): Pertains to an organism that is able to manufacture its own food.

Auxotrophic (Greek *auxein*, to add to plus *trophe*): Refers to mutants, generally of micro-organisms, which grow in culture only when specific requirements are added to the culture medium.

Bacteriophage (bacteria plus Greek *Phagein*, to eat): Literally eater of bacteria; a virus that infects specific bacteria, multiplies there-in and ultimately destroys the host bacterial cell.

Base Plate: The hexagonal plate at the end of the helical tail to which the tail fibres are attached.

Binal (Latin *binarius*, two by two): Refers to bacteriophages with a combination of two different symmetries; for example, bacteriophages T₄ with a cuboidal head and a helical tail.

Binomial (Latin *binominis*, two names): In Biology, each species is indicated by two names, one of the genus to which it belongs, and its own specific name.

Biparental: Refers here to the progeny produced out of a cross between two different parental types.

Capsid: The protein coat of complete virus particles.

Capsomere: The individual protein units which comprise the capsid of viruses, particularly of the cuboidal ones.

Carcinogenesis (Greek *karkinoma*, crab): The process of establishment of cancer.

Chemotherapy: Treatment of disease using chemical substances.

Chlorosis (Greek *chloros*, plus Greek *osis*, meaning green and a decreased state respectively): Refers here to failure of chlorophyll development because of nutritional disturbance or because of infection by virus bacteria or fungi.

Chorioallantoic membrane (Greek *chorion*, the envelop of the embryo plus Greek *allas*, *antos*, a sausage): In embryos of birds, e.g., chick embryo, the chorion (outer covering of the embryo sac) is fused with the allantois (sac like outgrowth of ventral side of gut) forming a double membrane structure which has been extensively used for

cultivating viruses in the laboratory.

Clone (Greek *klon*, a twig): The aggregate of individual organisms produced asexually from one sexually produced organism. Here refers to the cells and tissues developed in culture.

Collar: The thin disc which serves as an attachment area between the head and the tail of a binal virus.

Complementary strand: Here refers to the newly synthesized strand of DNA utilizing a previously present strand as a template, thus forming a newly formed double stranded structure.

Complementation (Latin *complementum*, to fill): Here used to indicate a phenomenon relating to successful multiplication of a functionally incomplete bacteriophage only when it is associated with another functionally in complete phage. The latter acts as a 'helper'

Complex virus: Viruses with relatively complex architecture and also having a specific mode of development, e.g., poxviruses.

Contagium Vivum Fluidum: Latin phrase coined by M.W. Beijernick describing diffusible, filtrable and infectious suspension of tobacco leaves infected with tobacco mosaic virus. Literally means living infection fluid.

Copy choice: Refers to the mechanism of genetic recombination in bacteriophages. Relates to exchange of fragments of one viral genome with that of another viral genome, both simultaneously infecting the same host cell. This exchange takes place during replication of the nucleic acid molecules of both the viruses taking place at the same time in the host cell.

Core (Latin *cor*, the heart): The central nucleic acid portion of a virus particle.

Cuboidal (Latin *cubus*, dice): Refers to the category of viruses having a cube like morphological appearance.

Cyanophage: Viruses which infect blue-green algae and destroy them.

Dalton (After J. Dalton, British physicist): A unit for measurement of molecular weight of complex macromolecules; is equivalent to weight of 1 molecule of Hydrogen.

Defective phage: Indicates functionally incomplete bacteriophages, those which cannot complete the replication cycle.

Disjunction (Latin *disjungere*, to disunite or to separate): Refers to the dissociation of the component parts of a virus particle.

Diploid cell strain: Cultures of cells or tissues derived out of primary cultures (c.v.).

Dual host virus: Refers to viruses that can carry out the process of multiplication in the cells of two taxonomically distinct, unrelated and independent hosts, e.g., Kyasanur Forest Disease Virus (op.cit.).

Early Proteins: Proteinaceous substances, particularly enzymes, synthesised during the early stages of bacteriophage multiplication; are involved in various biochemical activities during phage multiplication.

Eclipse (Greek *eklipsis*, failure to leave or come out): The period during bacteriophage multiplication which starts with the injection of nucleic acid into the host cell till the formation of first complete phage particle.

Envelope (Old French *envelop*, to cover): The outer membranous lipoprotein structure covering the nucleocapsid particles of most animal viruses.

Episome (Greek *epi*, upon plus *soma*, body): Indicates the independently replicating genetical units that are often associated with the chromosome or the nuclear material of certain organisms. These are foreign in origin and are capable of imparting genetical changes in the host. e.g., temperate phages causing lysogenic conversion (q.v.).

Extracellular virion: Refers to complete infective virus particles as they exist prior to infecting a host.

Fibroblast cells (Latin *fibra*; thread plus Greek *blastos*, a sprout): A type of cells of irregular, branching shape found throughout vertebrate connective tissue; its function is to form and maintain collagen which is a kind of fibrous protein that acts as a cementing material for binding cells and tissues together.

Filtrable Agents: Refers here to agents smaller than bacteria as these are able to pass through bacterial filters. Originally used as a criterion for defining viruses.

'Flying pin': Refers to the mechanism of transmission of myxomatosis virus causing myxomatosis of rabbits by mosquitoes. Refers particularly to the mosquito.

g-Antigen: A particular kind of particles produced during multiplication of certain animal viruses; act as precursor to the complete virus particles. Consist of viral nucleic acid, haemagglutinin (q.v.) and lipids.

Glucosylation: Refers to the process of attachment of a glucose residue to the nucleic acid molecules of certain bacteriophage mutants. This renders the nucleic acid resistant to the influences of host cell nucleases, thus enabling the phage to infect hitherto resistant strains of the bacterium. Glucosylation is possible only when the phage previously infects a bacterium possessing the necessary enzyme system.

Graft (Latin *graphium*, to write or to imprint): Here refers to the artificial union between tissues that are normally separate. Also

refers to placing of a tissue from its normal position to another position of the same organism or to any position of a different organism. Grafting of a diseased tissue or a portion therefrom can help transmit the disease.

Head: Refers to the cuboidal component of binal (q.v.) viruses.

Haemagglutinin (Greek *haima*, blood plus Latin *agglutinare*, to glue together): Refers to agents capable of inducing sticking together of red blood corpuscles. This happens when blood of incompatible group come together; viruses act as agglutinating agent.

Heat-therapy: Refers to treatment of diseases by application of heat.

Helical (Greek *helix*, a spiral): Indicates here the spiral arrangement of capsid subunits surrounding the nucleic acid of particularly the cuboidal viruses.

'Helper' virus: A defective phage which can help another defective phage (q.v.) to make a successful infection, cf. *Complementation*.

Hexamer (Greek *hex*, six plus Greek *meros*, part): Here denotes the number of component protein units forming a capsomere.

Host induced modifications: Generally non-hereditary alterations in the nature of a virus induced by its host. For example, conversion of non-virulent phages into virulent ones after they pass through a host with glucosylating enzyme. The viral nucleic gets glucosylated (q.v.) and is thus able to infect hitherto resistant, nuclease (q.v.) possessing bacteria.

Hyperplasia (Greek *hyper*, excessive plus Greek *plasis*, a forming or growth): An increase in the amount or mass of tissue due to an increase in the number of cells which individually keep their usual size. Often occurs as a result of viral infection.

Icosahedron (Greek *eicosei*, twenty plus Greek *hedra*, seat): A solid structure with twenty quadrangular faces. Refers here to twenty faceted virus crystals.

Immunity (Latin *immunis*, not serving or exempt): Refers here to the ability of plants and animals to resist infection by parasitic organisms including viruses. This ability could be genetical or be acquired. There are various mechanisms explaining immunity, some general and some specific.

Immunization: Method of imparting or injecting an immunogen (q.v.) into a susceptible host organism.

Immunogen: An agent which can induce immunity in otherwise susceptible host; for example, inactivated virus particles.

Inapparent infections: Refers here to virus infections that remain hidden, i.e., are not expressed, at least for an indefinite period. Symptoms do not appear and the infective particles may be carried from

one generation to the other. Under certain conditions some factors may induce the hidden infections to become and cause the disease symptoms to appear.

Inclusion bodies: General name given to the characteristic particulate structures present in certain virus infected host cells. For instance, the Negri bodies present in nerve cells infected by rabies virus. These bodies act as diagnostic features.

Induction: (Latin *inducere*, to lead into): Refers here to the conversion of a temperate phage (q.v.) into a lytic phage (q.v.). It could be spontaneous or induced.

Interference. (Latin *inter*, between plus Latin *ferire*, to strike): Here refers to the intervention of successful viral infection, occurring as a result of a variety of reasons; for instance, failure of infecting particles to get adsorbed to the specific receptor sites on the host cell wall.

Interferon: A specific proteinaceous substance, first discovered from influenza virus infected cells. These substances interfere with the process of multiplication of the virus in the host cell. These are host specific; different types of interferons are known to be produced when different host cell types are infected with the same virus; their mechanism of action involves prevention of protein synthesis.

Kappa (Cuppa) particles: Self replicating (q.v.) cytoplasmic particles present in *Paramecium*; these are instrumental in cytoplasmic inheritance and are thought to be episomic (q.v.) in origin.

Latency (Latin *latens*, to be hidden): Here refers to the state of inapparent infection of a virus.

Latent period: This may be regarded as the penultimate stage of the replication cycle of the bacteriophages. This period begins with the formation of the first new phage particle in the host cell and ends with the lysis of the same (q.v.).

Late-proteins: Indicates the proteins synthesized during the later stages of the replication cycle. These are usually the structural proteins necessary for the formation of capsids (q.v.).

Localized (Latin *localis*, a place): A kind of infection which is localised or restricted to a particular tissue or organ of an organism. Does not spread throughout the entire body of the organism.

Lysis (Greek *lysis*, dissolution): A process of disintegration or destruction; refers specifically to the destruction of host cells (e.g., bacterial) during and on the completion of bacteriophage multiplication.

Lysogenic Bacteria (Greek *lysis* plus New Latin *genic*, to give rise to): Here used to indicate bacteria carrying a phage in its temperate

(q.v.) state; the phage may eventually lead to the destruction of the erstwhile host bacterium itself.

Lysogenic Conversion: The influences of the prophage (q.v.) DNA on the host bacterium carrying it. Such influences include change in pathogenicity and in morphological nature of the host.

Lysogeny: This term refers to the phenomenon where the prophage (q.v.) DNA remains associated with the host cell chromosome till it can be or is induced to get separated and act as a virulent (q.v.) phage to destroy the host cell itself.

Lysozyme (Greek *lysis*, dissolution plus *zyme*, yeast): Refers to an enzyme system capable of hydrolyzing bacterial cell wall. Bacterial in origin.

Maturation (Latin *maturus*, ripe): Here indicates the process during which the individual components assemble and form into mature particles; an essential feature of virus multiplication.

Monolayer (Greek *mono*, single): Uniformly thick, often single layered cultures of cells grown in the laboratory out of individual clones (q.v.).

Monomer: Usually referred to the simplest of chemical compounds having the same empirical formula; here indicates simplest of protein units comprising a capsomere (q.v.); normally constituted of one or two polypeptide chains.

Multimeric (Latin *multus*, many plus suffix *mer*): Refers to protein units formed out of several monomers and themselves comprising the capsomeres.

Necrosis (Greek *necros*, dead plus suffix *sis*): Refers here to a kind of symptom of pathogenic infection by a micro-organism, including viruses; characterised by death and consequent disintegration of the infected parts.

Negri Bodies (After Dr P. Negri, Italian scientist): Crystalline inclusion bodies formed due to aggregation of virus crystals in cells infected by rabies virus; named after their discoverer.

Neoplastic growth (Greek *neo* plus Greek *plasm*, body): Indicates tumorous growth arising out of abnormal localized multiplication of certain types of cells; *malignant*, if the growing cells infiltrate surrounding tissue and/or are carried by blood or lymph to other locations in the body, growing there more vigorously. Otherwise *benign*, i.e., localized at a particular place only and relatively less harmful.

Nucleases: A kind of hydrolytic enzyme (hydrolase), capable of hydrolysing nucleic acids (q.v.) into their constituent nucleotides, are of two types, namely, DNase (deoxyribonuclease) and RNase

(ribonuclease) hydrolysing DNA and RNA respectively.

Nucleic Acids: Compounds of pentose sugars, phosphoric acid and nitrogen containing base (purine and pyrimidine); these three units combine to form nucleotides which in their turn join to form polynucleotide strands, generally referred to as nucleic acids. These are of two types, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA); the former has ribose as the sugar and is generally single stranded, the latter has deoxyribose as the sugar and is generally double stranded. RNA is mostly found associated with cytoplasm and DNA with nucleus in a cell. Both the types are characteristic of cells of all living organisms. However, in viruses only, either of the types is observed. DNA is considered to be the universal genetical materials and is the repository of the genetic code. RNA plays an important role in translating this code in terms of proteins, particularly enzymes.

Nucleoid: The central nucleic acid portion of a virus particle. Also called a core (q.v.).

Oncogenic (Latin *oncos*, growth plus Latin *genic*, to lead to): Indicates agents capable of inducing cancerous growth.

Occult virus (Latin *occultus*, hidden): Refers to a hidden virus i.e., a virus causing inapparent infection (q.v.) and thus remaining latent (q.v.).

Penetration: Refers to the process of injection of the bacteriophage nucleic acid into the host bacterium.

Pentamer (Greek *pente*, five plus suffix *mer*): Capsomers (q.v.) formed out of five monomer (q.v.) units.

Persistent (Latin *persistere*, to stand through): Here refers to a virus that is capable of multiplying, and thus persisting, within the body of the infection carrier or the vector (q.v.) making it an indefinite source of infection.

Peplomer (Greek *peplos*, a covering, or an envelop): Indicates the units of envelop that covers some virus particles and which is usually a lipid membrane.

Phage (Greek *phagein*, to eat): Short form for bacteriophages (q.v.) in general. Other types of phages are also known, e.g., cyanophage (q.v.).

Phenotype (Greek *phanein*, to show): Refers to a type determined by visible characteristics, Opposite of genotype (q.v.).

Phenotypic mixing: A process during which phages (q.v.) of two different types, which are usually close mutants (q.v.) infect the same bacterial cell and induce the host to produce their respective components. Then as the multiplication cycle proceeds during assem-

bly, some components of one type get constituted into the other type. Thus, the progeny becomes phenotypically mixed. The process, however, is not heritable.

Phylogeny (Greek *phylon*, race plus Greek *genesis*, beginning): Indicates the history of evolution of a group of related organisms.

Plaque (Dutch *plak*, a flat piece of wood): Refers to the clear areas in the plate cultures of bacteria caused by bacteriophage infection and consequent lysis (q.v.).

Pleotropic (Greek *pleon*, many plus Greek *trope*, turning): Here refers to mutants with several phenotypic (q.v.) forms.

Primary culture: Tissue cultures (q.v.) derived out of cells taken directly from the source, a plant or an animal.

Prophage (pro, a prefix meaning before): Here indicates a non-infectious virion (q.v.) which multiplies with the growing bacteria but does not bring about lysis to the host cell; these remain associated with the bacterial chromosome and are in fact a stage in the life cycle of a temperate phage.

Prophylaxis (Greek *prophylaktikos*, a guard before): Procedures adopted to guard against the occurrence of a disease; preventive measures.

Prototrophic (Greek *protos*, first plus Greek *trophe*, food): Here refers to micro-organisms that are not fastidious and can easily grow in normal culture media; cf. autotrophic (q.v.).

Quarantine (Latin *quaranta*, forty): Refers to the compulsory period of isolation (forty days for a ship and hence the term) to prevent spread of infection; usually suspected or actually infection carrying elements, including patients, are subjected to the procedure.

Receptor sites: The specific sites on the host, particularly bacterial, cell wall to which the infecting virus particles get adsorbed (q.v.)

Recombination (prefix *re* plus Latin *combinare*, to join): Refers to the process of formation in offspring of gene combinations not present in either of the parents, but are formed as result of combination of both the parental genomes. In viruses, this involves exchange of fragments of replicating DNA molecules of two different virions simultaneously infecting one host.

Reconstitution (prefix *re* plus Latin *constitue*, to make): Here refers to the process of assembly of artificially disintegrated virus particles, such as TMV, into complete ones.

Release (Old French *relasseire*, to relax): Indicates the process of letting loose of mature virus particles from bacterial cells, disintegrated as a result of bacteriophage infection; equally applicable to other host-virion systems.

Replication (Latin *replicare*, to repeat): Refers to the process of multiplication of viruses. A process analogous to the process of reproduction in true living organisms.

Replicative form (RF): Refers to the newly formed DNA strand, complementary to the already existing single stranded DNA synthesised during the early stages of the replication cycle of the bacteriophage ϕ x174. The replicative form is synthesised using the phage DNA as template. It is primarily responsible for the formation of new phage components.

Resistance (Latin *resistere*, to stand against): It is the ability on the part of the host cell to naturally prevent the occurrence of infection from any source.

Restricted (Latin *restrictum*, to limit): Refers to the bacteriophage mutants (q.v.) that are capable of infecting only a few strains of their otherwise normal host bacterium. This is because of presence of the preventive nucleases in the bacterial strains. Restricted bacteriophage strains can acquire the ability to infect on being previously glucosylated (q.v.).

Sedimentation: (Latin *sedimentum*, to sit): It is the process of settling down of suspended particles due to the force of gravity.

Sitala (Sanskrit *sital*, cool): Hindu mythological goddess, thought to be responsible for causing smallpox. Origin obscure.

Susceptibility (Latin *susceptum*, to take up): Refers to the inability on the part of the host cell to prevent infection (q.v.).

Symptom (Greek *sympnoma*, associated with or the root of an adverse condition): Phenotypic (q.v.) and/or physiological manifestations of a successful invasion of a host by a pathogen.

Tail: Refers to the helical component, a virus with binal (q.v.) symmetry.

'Target Organ': Indicates the location of the initial infection by viruses, particularly animal viruses. After getting established, the infecting particles may subsequently spread to other regions in the body.

Taxonomy (Greek *taxos*, order plus Greek *nomos*, law): The science dealing with describing, identifying, naming and classifying organisms.

Temperate phage (Latin *temperare*, moderate or restrained): Refers to the bacteriophages (q.v.) which on entering the host bacterial cell, do not necessarily lyse the latter; usually it remains associated with the host bacterial chromosome. This association, though indefinite, is not permanent, and the phage DNA, once detached, may again behave as a virulent (q.v.) type.

Titre (French *titre*, a standard): Refers to the standard or stock suspension of virus particles in an appropriate medium.

Transduction (Latin *transducere*, to lead or to pass across): Here refers to the process of bacteriophage (q.v.) mediated gene transfer from one bacterium to another. Usually through the agency of a temperate phage (q.v.). Rarely, other host-virion systems are also involved.

Transmission (Latin *transmittere*, to send): Refers to the process of sending a substance from one location to another; particularly pathogens.

Ultra-centrifugation (Prefix *ultra* plus Latin *centrum*, centre and Latin *fugere*, to flee away from): Refers here to the process of sedimentation (q.v.) of suspended particles subjected to very high centrifugal force in a centrifuge (q.v.). Since different kinds of particles sediment at different rates, the process offers a convenient means for ascertaining the homogeneity of a suspension. Molecular weights and sizes of the sedimenting particles can also be determined from the rates of sedimentations.

Ultra-structure (Latin prefix *ultra*, extreme plus Latin *structura*, to build by assembling components): Refers to the extremely detailed analyses of the structure of a cell, its organelles and other components. Involves sophisticated techniques, such as X-ray crystallography and electron microscopy.

Un-restricted (Prefix *un* plus Latin *restrictum*, to limit): Refers here to bacteriophage mutants (q.v.) capable of infecting a variety of bacterial strains; cf. restricted (q.v.) types which can be induced to become unrestricted.

Vaccine (Latin *vacca*, cow): A suspension of weakened or killed viruses or any other pathogen which when injected into the body of a susceptible host, immunizes (q.v.) the latter, against the same type of pathogen, or even against their toxins.

Vaccination: Refers to the process of introducing vaccine (q.v.).

Vector (Latin *vector*, carrier): Indicates an organism, usually an insect, that carries and/or transmits disease causing organism.

Virion: It is a virus (q.v.) particle that is fully infective.

Virus (Latin *virus*, a poisonous or slimy fluid): These may be defined as obligately parasitic, self replicating, non-cellular organisms, composed essentially of a protein covering surrounding a central nucleic acid molecule that is either DNA or RNA; often cause diseases like, smallpox or influenza.

Virulence (Latin *virulentia*, a stench): Refers to the relative infectivity

of a pathogen. Often in terms of its ability to overcome the resistance (q.v.) offered by the host.

Zymophage (Greek *zyme*, yeast plus *phage*): Virus infecting and destroying yeasts.

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AN INTRODUCTION TO VIRUSES

Third revised edition

S.B. Biswas and Amita Biswas

The book aims at providing a general overview of viruses at an elementary level and presents viruses as a broad group of highly specialized organisms, not necessarily to be compartmentalized in accordance with their hosts.

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